“I keep six honest serving-men
(They taught me all I knew);
Their names are What and Why and When
And How and Where and Who.”

From The Elephant’s Child (1902) by
Rudyard Kipling (1865 - 1936).

University of Alberta

OBSERVATIONS ON ICHNOLOGY, TAPHONOMY AND EPIBIOTA IN THE FRESHWATER REALM

By

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DEDICATION

This work is dedicated to all the men who have challenged me to bring forth the best version of myself, who have provided me with unwavering support and who have held faith in my abilities and potential, even through those times when I have doubted myself.

Most of all to my father, Dr Matthew Francis Martin Lawfield.

ABSTRACT

Ichnology concerns the study of interactions between organisms and both soft and hard substrates. Actualistic observation of a modern day river channel molluscan assemblage including unionid and sphaeriid bivalves and gastropods within the Saint John River, Fredericton, New Brunswick, Canada reveals their production of almond shaped *Lockeia* like resting traces, together with varied, horizontally aligned furrowed, meandering, looping and spiral plan view locomotion/grazing traces. These traces occur within a shifting sand softground substrate.

An emersion event associated with a low water level allowed collection of unionid samples, amongst which *Elliptio complanata* predominates, alongside *Lampsilis radiata* and *Anodonta implicata*. Detailed analysis of shell material, with methodologies including scanning electron microscopy (SEM) reveals microboring, various taphonomic signatures and the development of biofilm and extracellular polymeric substances (EPS). These are often associated with one another and their location closely influenced by the shell structure.

Taphonomic decay of the shells was noted, with the external periostracum and prismatic aragonite layers decaying, particularly in the umbonal region. Physical puncturing and tearing penetrate the periostracum. Exposed regions of nacreous aragonite are smoothed by a combination of chemical interaction with the water column and physical abrasion from agitated silt and sand sediments. Surface pitting and circular to ovoid penetrations with morphologies comparable to *Oichnus* borings are noted and their formation is tentatively attributed to bacterial decay processes.

In marine settings, the outer protective periostracum of molluscs often possesses physical and chemical defence mechanisms intended to inhibit the attachment of epibiota. Our observations suggest their absence from unionids, with final instar larval or pupal stage Trichoptera, including *Goera*, *Neophylax* and *Helicopsyche* selectively attached to regions of intact periostracum in preference to exposed aragonite on dead shells. In addition, live unionids can display thick growths of cyanobacterial or cyanophyte dominated microbial mat. Biofilm and extracellular polymeric substances, with bacterial, diatomaceous and filamentous components are also observed, often displaying a close association with both microboring and the shells conchiolin layers. Several styles of microboring are noted, with predominantly surficial and both simple tubular and complex network penetrative styles observed. Microborings may be attributed to cyanobacterial, cyanophyte and fungal activity.

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LIST OF SYMBOLS, NOMENCLATURE, OR ABBREVIATIONS

ABO	Accessory Boring Organ
EDX	Energy-Dispersive X-Ray Spectroscopy
EPS	Extracellular Polymeric Substances
PAR	Photosynthetically Active Radiation
SEM	Scanning Electron Microscopy
SLR	Single-Lens Reflex
sp.	Species
UV	Ultraviolet

CHAPTER 1

INTRODUCTION

INTRODUCTION

From its origins through to the present day, the history of life on this planet has been dominated by aquatic organisms. Initially life developed in marine settings, subsequently extending to freshwater environments, either directly or via secondary invasion of the freshwater aquatic realm by terrestrial intermediaries. The freshwater realm presents unique challenges (e.g. Miller and Labandeira, 2002) that both delayed and inhibited its exploitation to the full extent observed in marine settings. Amongst the more successful freshwater fauna are the unionid bivalves, members of the molluscan phylum that are commonly referred to as freshwater "clams". These organisms form the focus of this thesis, which presents detailed observations regarding the interactions of unionids with the soft sediment substrates they inhabit, as well as a variety of means by which the unionids themselves serve to provide a substrate for the activities of other organisms.

This thesis is interdisciplinary in nature, being primarily concerned with the exploitation of substrates by organisms, illustrated principally with examples from within the domain of ichnology, but also via epibiotic associations. The study addresses substrate exploitation at a number of scales, providing examples from freshwater and more specifically river (fluvial/lotic) locations. Examples are provided from soft sediment occurrences, however most of the examples are drawn from hard shell substrates and in particular those provided by unionid bivalves. Organisms observed to exploit the unionids shell surface as a habitat are noted and the structures they create both on and within the shell are described.

These interactions are diverse and include examples of epibiota, including bacteria, diatoms, biofilms and microbial mats as well as caddisflies. In addition, the taphonomic degradation of the unionid shell surface is approached in the context of physical, chemical and biogenically mediated mechanisms of decay. A complex interplay is noted between the distinct layers of the shell structure, taphonomic processes, passive colonisation of exposed shell surfaces and the creation of boring structures. As a result of this complexity, with a number of independent processes being observed to act simultaneously, it is impossible to examine one aspect of these systems in isolation. To overcome this, a broad, holistic and interdisciplinary approach to their study has been adopted here, with individual chapters highlighting various aspects of these processes and associations. Although some of these areas had received a peripheral or cursory mention in the existing published literature, detailed study of these associations had not been presented before.

This introductory chapter provides brief overviews of the most pertinent aspects of the varied disciplines that are addressed in the body of the thesis, providing a theoretical background and context for the more detailed chapters that follow. In addition, this chapter highlights the main areas of knowledge advanced by the study and provides a brief outline of the topics addressed in each of the subsequent research chapters.

BACKGROUND

Brief Review Of Ichnology

Ichnology is that branch of scientific endeavour concerned with the study of structures produced by the interaction of living organisms with a substrate. The substrate may be either soft sediment, of varying degrees of firmness, or a hard substrate in the form of rock, wood, bone or shell material. Structures associated with soft substrates include tracks and burrows, whilst those in hard substrates are referred to as borings. The majority of the literature associated with these structures addresses preserved rock record examples (ichnofossils or trace fossils). The study of recent, modern day examples can also be undertaken (neoichnology), having the potential to provide valuable information about the causal organisms and their behaviour (ethology), thus serving as a useful aid to the interpretation of preserved structures. The structures created by an organisms behaviour may often be considered as examples of an “extended phenotype” (Dawkins, 1982; Hunter, 2009). Ichnology, by its very nature is a syncretic science, bridging the margins of and drawing from sedimentology, palaeontology and biology. As such it represents the closest branch of modern science to the traditionally broad field of natural history as employed by Darwin, amongst other early observational scientists.

Chronologically, ichnological literature was initially dominated by taxonomic papers, with the later addition of ethological papers and subsequently the development of the Seilacherian ichnofacies concept as a unifying model that groups distinctive trace assemblages associated with particular environmental parameters (e.g. see recent reviews in Baucon et al., 2012; MacEachern et al., 2012). Observations presented in chapter two describe a modern example of a lotic freshwater fluvial river channel trace assemblage that displays some overlap between currently established freshwater invertebrate ichnofacies.

Ichnology in non-marine, freshwater and specifically fluvial settings has been subject to a number of broad reviews (e.g. Buatois and Mángano, 2004, 2007; Melchor et al. 2012; Scott et al., 2012 and references therein). Overviews of nonmarine aquatic traces are provided in for example Chamberlain (1975) and Hasiotis (2002). The range of ethologies that may create visible traces parallels that for marine settings, with resting, crawling, dwelling and feeding traces of bivalve and gastropod molluscs being reported (Chamberlain, 1975) as well as bivalve escape traces that record upward movement of bivalves to avoid their being deeply buried during rapid sedimentation (Thoms and Berg, 1985). In addition to these molluscan examples, the aedifichnia (traces constructed from raw materials, as described by Donovan, 1994) of caddisflies also occur in freshwater settings. Despite this diversity, there remains a paucity of detailed ichnological studies addressing river settings across either modern assemblages or from rock record locales.

In addition to the aforementioned soft substrate traces, hard substrates may provide a setting for boring activity which may provide evidence for the existence of organisms including soft bodied fauna whose remains would otherwise not be preserved. Organisms with a boring habit are a well established feature of marine environments, with microboring being “ubiquitous in carbonate substrates of the illuminated seafloor today” as a result of the activities of “endolithic photoautotrophic microorganisms (algae and Cyanobacteria)”. In addition, “chemoheterotrophs (mainly fungi) occur in virtually all marine environments” and “fungal borings are common over a broad bathymetric spectrum” (Bromley, 2004). Representative examples from within many of the broad taxonomic groupings of organisms responsible for boring in saline waters also occur in freshwater environments. The variety of selection pressures that might potentially favour the adoption of a boring habit by microorganisms are reviewed and assessed in Cockell and Herrera (2008) and references therein. These pressures may include some combination of the acquisition of limiting nutrients contained in the substrate, protection from harmful ultraviolet (UV) radiation and extremes of photosynthetically active radiation (PAR), protection from desiccation and extreme temperatures, space competition, prevention of detachment by water currents, as well as avoidance of both grazing predation and entombment by mineralisation. It may be assumed that microboring organisms in freshwater settings are presumably subjected to similar selection pressures.

Brief Review Of Freshwater River (Lotic) Ecosystems

The origins, longevity, ongoing complexity and importance of freshwater ecosystems (a community of organisms and the environment they interact with) are recounted by Miller and Labandeira (2002) and Gray (1988). These authors emphasise the much greater variation in both physical and chemical properties observed in freshwater environments in comparison with marine settings. Lotic (flowing water, e.g. stream and river) systems are thought to have played a significant role as a bridging route enabling the evolutionary colonisation of terrestrial habitats by formerly marine fauna (Thorp and Covich, 2001). Lotic environments offer several advantages for colonisation in comparison to lentic (still water, e.g. lake and swamp) systems. As summarised in Thorp and Covich (2001), these include greater turbulence and helical flow leading to an absence of stratification, generally higher oxygenation, smaller temperature fluctuations and more even distribution of nutrients and plankton. They are also more permanent. Despite their prominent position both geologically and biologically, lotic systems are comparatively underrepresented in studies of freshwater systems in comparison to lentic systems (Thorp and Covich, 2001).

Dissolved oxygen concentration, salinity and pH are the main chemical controls on invertebrate distribution in lotic settings (Thorp and Covich, 2001). Physical controls on distribution are also extremely important, with substrates exerting a significant influence on the lives of invertebrate faunas. Benthic organisms in particular, which represent the majority of invertebrates found in freshwater settings interact with substrates for a variety of ethological reasons including: “resting, food acquisition, reproduction, and development (e.g. places for pupal case attachment)”. Physical substrate parameters are also important in so far as they may provide refugia from predation or inhospitable physical conditions (Thorp and Covich, 2001). As a result of the variation in these influencing factors, both the abundance and diversity of fish and molluscs are generally found to increase with stream order, however the opposite trends are noted for insects (Thorp and Covich, 2001). More local variations are also important and Thorp and Delong (1994) observe that “Invertebrates in general tend to be clustered in nearshore (especially on rocks...), shallow water regions of rivers...where they have access to both benthic algae and allochthonous material entering from the riparian zone.” The grain size of the substrate is an important influence on the distribution of organisms and Wallace and Anderson (1996) remark on the unfavourable nature of river and stream sand substrates for exploitation by aquatic insects, owing to an absence of attachment sites and a general paucity of food resources.

Brief Review Of Unionid Bivalves

The biology and ecology of freshwater bivalve molluscs in general and unionid bivalves in particular has been reviewed by several authors, with McMahon and

Bogan (2001), Strayer (2008), and Cummings and Graf (2010), providing some of the more recent and comprehensive summaries. Unionid bivalves have a global distribution, with modern examples present on all continents outside the Antarctic (Strayer, 2008). They are at their most diverse in North America, where they are represented by nearly 300 species (Lydeard et al., 2000). Unionid bivalves are an important component of freshwater ecosystems (e.g. Strayer, 2008; Cummings and Graf, 2010), where they play a significant role in a number of ecosystem processes and often dominate the benthic invertebrate biomass (McMahon, 1991).

Although their evolutionary roots and ancestral environment are marine, the geological record indicates that bivalves have made several incursions into the freshwater realm, overcoming the major challenges associated with colonisation of this new environment. Amongst the more significant obstacles faced by this transition are those relating to osmoregulation and the marked daily and seasonal temperature fluctuations, both of which are avoided in marine settings (e.g. Miller and Labandeira, 2002). Of those molluscan taxa that have successfully made this transition and that are still extant at the present day, amongst the longest established are unionid bivalves, which occur in both modern lotic (moving water, e.g. river) and lentic (standing water, e.g. lake) habitats, in addition to being preserved in fluvial and lacustrine sedimentary rocks. Unionids may possess a fossil record dating to the Devonian (e.g. Chamberlain, 2004; Chamberlain et al., 2004) and are certainly established by the Triassic (Watters, 2001). The origins and palaeogeographical distribution of unionids are addressed in more detail by Gray (1988), Taylor (1988), Watters (2001), Strayer (2008), and Cummings and Graf (2010).

A full and comprehensive discussion of the life cycle and ecological preferences of unionid bivalve faunas is beyond the scope of this chapter, with pertinent aspects being addressed within the introductory and discursive material of individual chapters. Briefly however, unionids are exclusively freshwater bivalves. They occur in both rivers and lakes, but are more diverse in the former. Although hermaphroditic examples occur, two sexes are more usual. Reproduction occurs by fertilisation through sperm broadcast into the water column. Next, in the majority of species, an obligate ectoparasitic glochidial larval stage occurs. This involves fertilised eggs developing into a specialised glochidial developmental stage, these glochidia being stored in marsupia pouches on the gills of the mussel. When this stage occurs, the females are termed gravid (e.g. Bauer, 2001A; Wächtler, 2001). Following their release from the unionid, the glochidia attach to fish, generally and most successfully in the gill region. Here they are encysted and continue their growth before breaking free and entering the substrate of the river bed sediment where further growth occurs. This allows for efficient dispersal throughout the geographical range of the host fish. Preference for a limited range of host fish is frequently exhibited (Wächtler, 2001). The origins of this relationship between bivalve and host fish and it's significance for palaeogeographical and modern ranges is assessed by Watters (2001) who infers a mesozoic origin for the relationship based on the origins and distribution of freshwater teleost fish which are generally the preferred modern hosts. This chapter presents many generalities that may be assumed to apply to the majority of unionid taxa and populations, however there is a wider range of life histories than suggested here and this variation is highlighted in Bauer (2001A) and Hochwald (2001). In addition to the ranges of their host fish, the distribution of unionid bivalves is governed by a number of physical, chemical and biological factors, with a preference being exhibited for a stable, supportive and non-clogging substrate type. A range of other factors that include water velocity and depth, food availability, oxygenation, temperature (e.g. Hornbach, 2001; Strayer, 2008 and references therein), geology, and vegetation (Strayer, 2008 and references therein) are also considered significant. Optimal conditions for juveniles and adults may differ, further complicating a comprehensive understanding of which factors are most significant (Strayer, 2008).

Adult unionids are free living, benthic and commonly sedentary. Feeding is by a combination of filter feeding upon a wide variety of seston including bacteria, phytoplankton, zooplankton and organic detitus (e.g. discussion in Strayer, 2008 and references therein) and pedal deposit feeding. Filtration by dense populations may affect water clarity (e.g. Pusch et al., 2001). Depending on species, an endobenthic or epibenthic mode of life may be adopted, with preference for full burial being associated with harsh environmental conditions. Many species are noted for their longevity, with decadal age ranges commonly noted for most species, particularly in the warmer waters of temperate regions. Even longer ages, often exceeding a century, are reported for populations of *Margaritfera* in colder northern waters (Bauer, 2001B; Mutvei and Westermark, 2001, and references therein).

Unionids are of considerable ecological significance (e.g. Vaughn, and Hakenkamp, 2001), playing a significant role in habitat creation as habitat engineers (Gutierrez, et al., 2003), as well as sequestering calcium carbonate (Green, 1980; Strayer and Malcolm, 2007). The presence of unionid bivalves in an invertebrate assemblage bespeaks of an ecosystem in fine fettle, since their ecology is closely tied to both planktonic food sources and the presence of a fish hosts for their obligate ectoparasitic glochidial larval stage that in most species is a compulsory part of their lifecycle. In addition to these biotic interactions, unionids are susceptible to damming and other physical alterations to habitat, as well as chemical pollutants (Strayer, 2008; Cummings and Graf, 2010; Schwalb et al., 2011). Commercial exploitation has reduced their numbers and led to local extirpation of certain species in some areas of their historical North American distributions. More recently their populations have been further reduced and locally threatened by degradation from pollution and modification of the lake and river habitats where they occur and also, in the case of both European and North American populations, by the introduction of invasive populations of fouling encrusters (e.g. Zebra Mussel, *Dreissena polymorpha* and Quagga Mussel *Dreissena rostriformis bugensis*) and competing (e.g. Asian Clam *Corbicula fluminea*) freshwater bivalves, that, where present, have had a significant impact on endemic populations (see recent reviews in Miehls, 2009; Sousa et al., 2011; Benson et al., 2013A for *Dreissena polymorpha*; Benson et al., 2013B for *Dreissena rostriformis bugensis*; Sousa et al, 2008; Foster, 2013 for *Corbicula fluminea*; Cummings and Graf, 2010, for all species). The combination of these factors has led to numerous extinctions and the endangering of remaining faunas (e.g. Strayer, 2008). In addition to these stressors, the effect of parasites and disease, predation may occur by a variety of invertebrate fauna predating juvenile unionids. Predation by vertebrates including otter, raccoon, muskrat, fish, birds and turtles also occurs, targeting either juvenile or adult unionids (e.g. Strayer, 2008 and references therein).

In archaeological and ancient contexts as well as amongst some indigenous communities unionids represented a significant human food resource (e.g. Parmalee and Klippel, 1974; Walker et al., 2001; Wolverton et al., 2010; Valledor de Lozoya and Araujo, 2011). Historically they have also been of broader economic significance, being exploited as tools by first nations communities (e.g. Tennant, 2010). Industrial societies have exploited unionids as a source of freshwater pearls and buttons, as well as providing feed for poultry and pigs, through to the mid twentieth century (Coker, 1919). More recently their fragmented shells have been used for seeding cultured marine pearls (Cummings and Graf, 2010; Thorp et al., 2010).

The higher level taxonomy of Unionid bivalves is currently in a state of flux as molecular analysis is being used to refine and in some cases overturn existing classifications (Graf and Cummings, 2007). At the species level, visual sample identification may be complicated by the unionid bivalves property of exhibiting ecophenotypic plasticity (Bauer, 2001A) that gives rise to variable external morphologies, based on local environmental conditions. Traditionally the order Unionoida includes the superfamilies Etherioidea and Unionoidea, the latter of which is subdivided into the families Unionidae, Margaritiferidae and Hyriidae. The families Unionidae and Margaritiferidae include a North American distribution (e.g. summaries in Hoeh et al., 2001; Strayer, 2008; Cummings and Graf, 2010). All of the unionid species we encountered in our study belong to the family Unionidae. The Identities of the unionid and broader molluscan fauna reported in this thesis were confirmed visually by reference to Clarke (1981), using a combination of both internal as well as external shell features wherever possible, in addition to their known ranges of geographical distribution.

As this thesis shows, unionid bivalves can provide an interesting variety of ichnological and related phenomenon. The bivalves themselves interact directly with their surrounding substrate, giving rise to a variety of distinctive trace patterns, occurring alongside structures produced by other freshwater molluscs (see chapter two). The layered shell structure, with both aragonitic and organic proteinaceous conchiolin layers influences taphonomic processes operating on the shells (chapter three). The chemically and physically varied bivalve shell also provides a valuable habitat space that is noted to be exploited for attachment of other organisms (chapters three to six) and also as a substrate for microboring activity (chapter four).

Brief Review Of Epibiota

Within the aquatic realm there are a number of selective advantages that accrue to organisms that are able to exploit hard substrates (e.g. Wahl, 1989 and 2010 for extensive explanations and revues associated with the marine realm). As a result of these benefits, available substrates are rapidly colonised. The availability of appropriate substrates is often a limiting factor in the expansion of populations of organisms that exploit these habitats and as a result both dead and live substrates may be exploited, with organisms that attach to live, biogenic substrates being referred to as epibiota. Epibionts may be literally defined as “organisms that live on the surface of organisms” from the Greek root prefix epi meaning above or on and biont meaning organism. Epibiotic associations are an oft-ignored component (e.g. Lescinsky, 1996) of both modern ecological communities and fossil assemblages. Although their presence has previously been noted in freshwater ecosystems (e.g. see references in chapters five and six) the majority of studies addressing both modern and fossil record epibiota are directed toward the marine realm (e.g. Wahl, 1989; Lescinsky in Briggs and Crowther, 2001; Dürr and Thomason 2010, and references therein). When noted, the interpretation of such associations provides a significant source of information regarding ecological relationship within a given community.

A number of selective advantages and disadvantages may accrue to the live substrate (basibiont) when epibiota are attached (Wahl 1989 and 2010). The disadvantages presented to the basibiont often slightly outweigh the benefits of these associations, however the presence of epibiota will generally be tolerated provided the balance of potential energetic costs and benefits associated with investing in defensive measures are outweighed by the benefits of investing the same energy in somatic growth. To prevent their exploitation, a number of strategies may be adopted by potential basibiont fauna to inhibit colonisation of their outer surfaces (e.g. Scardino et al., 2003; Bers and Wahl, 2004; Bers et al., 2006 and 2010 and references therein). These defences include both physical mechanisms, such as the presence of regularly spaced micron scale ridges on outer shell surfaces that serve to hinder or even prevent the settlement of larval stage epibiota. A number of chemical mechanisms may also inhibit larval attachment. Whilst such strategies are widely reported in the marine realm, their adoption or absence in freshwater settings does not appear to have been previously studied. Observations presented in chapters five and six suggest that such mechanisms are not present in the case of unionid bivalves, despite the presence of a variety of epibiotic organisms in the freshwater realm that exploit unionids.

The importance of unionid bivalves as a substrate for epibiotic organisms has been reported by Beckett et al. (1996), who note the exploitation of unionid substrates by Cnidaria, Turbellaria, Oligochaeta, Isopoda, Hydracarina, Ephemoptera, Trichoptera, Coleoptera, Diptera and Bivalvia as well as “aufwuchs”. They concluded that for their assemblages, site selection appeared to be purely a function of providing a hard substrate and was not due to some unique association with unionids. Other studies describing epibionts on unionids include that of Vaughn et al. (2002), in which river weed was observed on the posterior and around the siphon. Vaughn et al. (2008) noted increased levels of periphyton inhabiting the shells of live unionids in comparison with empty shells. Grazing invertebrate taxa including caddisfly, mayfly and midge larvae as well as mites and tardigrades all displayed a corresponding increase. Observations presented in chapter five and six expand on this foundation.

Detailed observations undertaken by Cox (1988 and references therein) on diatom assemblages attached to both plant and sediment substrates indicate that diatom distribution is closely related to the nature of the substrate, with nutrient availability being a significant factor. Cox (1988) suggests that studies of substrate specificity are often approached at an inappropriately coarse scale, contending that SEM observation should be more frequently employed and detailed patterns of distribution noted. In chapters three and four we present detailed observations on the distribution of components including diatoms, bacteria, biofilm and associated extracellular polymeric substances as well as their relationship to the chemically and physically distinctive unionid shell layers.

Brief Review Of Preservation Potential Of Association

Reviewing the preservation potential of the association described herein, the molluscan assemblage itself appears to have the greatest chance of transitioning to the rock record, as preserved body fossils, and indeed, a similar molluscan fauna has been reported by Johnston and Hendy (2005) from the Upper Cretaceous of Dinosaur Provincial Park, Alberta, Canada. At that location, two distinctive assemblages occur, one comprising elements with a variety of gastropods, including *Campeloma*, found in association with sphaeriid bivalves and rare unionids. In addition a separate assemblage composed predominantly of unionids, which are “often articulated and in life position” occurs, found in association with the trace fossil *Lockeia* and vertical escape traces. Johnston and Hendy (2005) report marked local lateral spatial variation in the distribution of the two distinct assemblages, giving rise to dramatic variability in the densities of both the molluscs and the traces and this accords with our observations on the distribution of modern molluscs and traces. Further examples of unionid fossil preservation are noted and reviewed in chapter three. Soft sediment trace fossil assemblages that are similar to ours are reported from a variety of preserved lacustrine deposits, although, as reviewed in chapter two, fluvial deposits are usually associated with a different assemblage of trace fossils (e.g. Lawfield and Pickerill, 2006; Melchor et al, 2012, and references therein). There appears to be no published research addressing freshwater microborings in the fossil record. Similarly, no published studies address freshwater microbial mat preservation. The only published example of a preserved freshwater epibiotic association we are aware of is described in Erickson (1983), associated with concretion growth preserving attached Trichoptera on a Palaeocene unionid bivalve. Assuming survival of the bivalve assemblage through to shallow burial, subsequent recrystalisation of the aragonitic unionid shell would presumably reduce the probability of any associated microborings being preserved. In addition, as noted in later chapters, a variety of processes including sloughing by sediment and loss of periostracum would tend to inhibit preservation of any epibiotic organisms associated with the molluscan assemblage.

RESEARCH AIMS

This research project aims to highlight the significance of freshwater molluscs and in particular unionid bivalves as agents of trace formation in soft sediment ichnology. In addition, the interplay of various taphonomic decay processes that operate upon the shells and the significant influence of the layered shell structure upon these are examined. The ecological significance of unionid bivalves is emphasised, with close relationships being demonstrated with a variety of microborings and epibiotic organisms that exploit the unionids shell structure as a substrate.

This study thus aims to expand upon existing knowledge in three key areas:
1) Freshwater ichnology, addressing both soft sediment traces and hard substrate microboring assemblages.
2) Early stage taphonomic processes affecting freshwater unionid bivalve assemblages.
3) Freshwater epibiotic associations at both microscopic and macroscopic scales.

THESIS OUTLINE

Readers should be aware that as a necessary consequence of the combination of shared sample collection location, in concert with the paper based format employed in this thesis, there is, inevitably, some overlap between the introductory material for a number of the thesis chapters. Similarly, some of the observed features are considered to be of significance in multiple chapters and are thus approached separately for both their description and interpretation from several angles with different aspects being emphasised depending on the subject of the individual chapter (for example the damage to and loss of the outer layer of unionid shell periostracum is noted or described in several chapters, both as a taphonomic process with reference to the mechanisms involved in its removal, as well as for the exploitable habitat space that is created, revealed and made available to microorganisms once this layer has been removed). An outline summarising the relationship of the submitted chapters is provided below, to aid and guide the reader.

Chapters two through six are closely thematically linked, outlining as they do the results of observations undertaken on and pertaining to a modern unionid bivalve assemblage from the Saint John River, Fredericton, New Brunswick, Canada. Both field and lab based observational techniques are employed, including scanning electron microscopy (SEM) imaging. The nature of the sample collection locality is addressed in terms of its climatic, hydraulic and sedimentologic situation. The presence and composition of the molluscan fauna present at the site is noted and details of a soft sediment trace assemblage are recorded. The significance of the bivalve shell as a substrate for other organisms is assessed next, across several chapters, and with a variety of taphonomic signatures, microborings and epibiota being described.

Chapter two provides an in depth description of a trace assemblage present in a lotic freshwater river (fluvial channel) sand-softground. The assemblage is dominated by a variety of molluscan generated looping and meandering locomotion/grazing trace forms. The traces embody a transitional ichnological assemblage, unusual in comparison with the traditional published freshwater ichnofacies models in constituting a composite assemblage displaying some overlap between elements conventionally associated with both *Mermia* and *Scoyenia* ichnofacies.

Chapter three outlines observations of the taphonomic processes that operate on unionid bivalve shells, illustrating the progressive decay with a series of samples from near pristine fresh shell, to substantially eroded shells exhibiting advanced decay. Taphonomic decay of the umbonal shell region is particularly prevalent. Areas of pitting are also shown, and these include morphologies that can closely resemble the predatory boring *Oichnus*. Possible mechanisms for the shell decay are discussed and evaluated, including the physical puncturing of periostracum and decay of newly exposed aragonitic shell layers that display evidence for a variety of physically, chemically and biologically mediated taphonomic decay processes.

Chapter four continues to further examine the theme of boring activity, in this case looking at microborings. A variety of structures are noted, some of which display a close relationship to the organic conchiolin layers present within the nacreous portion of the shell. The significance and extent of biofilm and extracellular polymeric substance (EPS) development are also reviewed, with their relationship to both the conchiolin layers and microborings being discussed. Bacterial, diatomaceous, and fibrous elements that may be attributed to a fungal, cyanobacterial or cyanophyte origin are all locally present in association with the biofilm.

Chapter five addresses the development of a thick epizoic microbial mat (early development of which often rests on a foundation of biofilm growth) found on the exterior shell surface of some of the live unionids. The limited extent of growth, which doesn’t appear to hinder the normal life functions of the unionids is assessed in terms of the ecological significance of this relationship.

Chapter six examines dead unionid shells as a substrate for larval Trichoptera (Caddisfly) attachment. The chemical and physical properties of the exposed outer shell structure clearly have a significant impact on selection of attachment sites, with the taphonomically exposed aragonitic portions of the shell being universally avoided, a preference being instead displayed for the pristine intact outer periostracum layer. This relationship is in marked contrast to epizoic attachment patterns adopted in marine fauna, where basibionts often possess adaptive features that evolved to inhibit the attachment of epibiota.

Chapter seven provides some concluding remarks, serving both to summarise the main findings of the individual thesis chapters and also relating the individual chapters to one another. In addition, limitations of the study are noted and suggestions for avenues of future research are explored.

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CHAPTER 2

A NOVEL CONTEMPORARY FLUVIAL ICHNOCOENOSE: UNIONID BIVALVES AND THE *SCOYENIA–MERMIA* ICHNOFACIES TRANSITION[1]

INTRODUCTION

Over the previous two decades, there has been a burgeoning interest in the field of continental invertebrate ichnology, with the well established Seilacherian *Scoyenia* ichnofacies (Seilacher, 1967, 1978; Frey et al., 1984; Bromley, 1996) characteristic of periodically emergent lacustrine, sheetflood, or fluvial situations, being amended and complemented by the lacustrine and floodplain pond *Mermia* (Buatois and Mángano, 1995, 1998) and non-aquatic *Termitichnus* (Smith et al., 1993; Genise et al., 2010), *Coprinisphaera* (Genise et al., 2000) and *Celliforma* ichnofacies (Genise et al., 2010) (see Keighley and Pickerill, 2003; Buatois and Mángano, 2004, 2007; Melchor et al., 2012; Scott et al., 2012 and references therein for recent reviews). Additionally the *Skolithos* ichnofacies, typically more characteristic of marine situations, may be encountered in the high energy setting of active fluvial channels and within high-energy zones in lakes (Buatois and Mángano, 1998, 2004). Despite the relative abundance of fluvial compared to lacustrine successions, the fluvial realm remains relatively underrepresented in both the modern and ancient ichnological record, with few described localities (e.g., Thoms and Berg, 1985; Pickerill, 1992; Sarkar and

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[1] *A version of this paper has been published. Lawfield and Pickerill 2006. Palaios. 21: 391-396.*

Chaudhuri, 1992; Melchor et al., 2012 and references therein) and no exclusive recurrent ichnofacies comprising fluvial channel facies. This is largely a function of taphonomic bias and cannot be taken to indicate the absence of trace making organisms and structures formed by them from within rivers. The majority of described fluvial traces, both modern and ancient, have been described from sheetflood, overbank, or abandoned channel deposits because continued reworking of active channel sediment generally precludes preservation in this setting. Despite a long running occupation of freshwater settings, few known fluvial trace assemblages have been attributed to a molluscan progenitor—bivalve traces being reliably identified from Devonian localities in New York and possible sites in New Jersey, Wales, and Ireland (Bridge et al., 1986; Chamberlain, et al., 2003, 2004) as well as the Cretaceous of Alberta (Johnston and Hendy, 2005). In addition to these examples from the preserved sedimentary record, the trace making activity of a modern bivalve and gastropod assemblage inhabiting a point bar setting was documented by Pryor (1967).

This chapter documents a modern example of a fluvial trace assemblage recorded from a sandbar adjacent to Jewett Island (45°58′N, 066°42′W), in the Saint John River, immediately upstream of Fredericton in New Brunswick, Canada (Figure 2.1). The assemblage displays features characteristic of both the *Scoyenia* and *Mermia* ichnofacies, and, as such, demonstrates their ethological continuity. Active trace making behavior was observed in a unionid bivalve dominated benthic molluscan community, after an environmentally stressful emersion event created conditions favorable for observation of the trace making organisms.

STUDY AREA

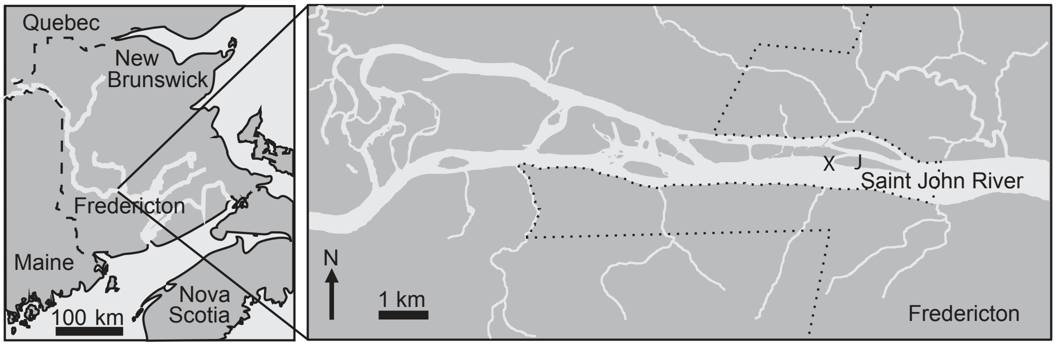

Figure 2.1. Locality map, depicting the study site (X) and Jewett Island (J) on the Saint John River, immediately west of Fredericton, New Brunswick, eastern Canada. The Mactaquac Dam occurs 2 km beyond the western figured limit of the main river channel. Dotted line = Fredericton city limits.

The Saint John River originates in the Maine Appalachians before flowing through Quebec and New Brunswick, eastern Canada, to the Bay of Fundy (Figure 2.1). The river has a low gradient (0.03% below the Mactaquac Dam, 13 km west of the study site), and, along most of its course, consists of a single, low-sinuosity channel. Immediately upstream of Fredericton however, the river displays an anastomosing aspect in plan view. To the west and upstream of the study site (Figure 2.2), the Mactaquac Dam creates an effective ecological barrier and sediment trap in addition to blocking tidal influence above the dam. Saline influence in the river is attenuated by the Kennebecasis Bay and Reversing Falls bedrock sills, located respectively 110 km and 120 km downstream from the study area. The river has a mean annual discharge of 1110 m^3/s, peaking during the April and May snow melt, despite evenly distributed precipitation (Cunjak and Newbury, 2005). The locality described herein represents a relatively high latitude assemblage (45°58′N, 066°42′W), with a humid continental climate exhibiting pronounced seasonality. Daily mean air temperatures range between -9.3°C in January and 19.2°C for July (Wikipedia, 2006). It should be noted that most descriptions of fluvio-lacustrine trace assemblages rarely take into account the

Figure 2.2. Study site, with the vegetated Jewett Island composed of stable Pleistocene sediment distal to a recently exposed sandbar on which traces were observed.

paleolatitude and paleoclimatic range of the settings they describe, although Good (2004) attempted a climatic reconstruction based on bivalve growth bands and Martin (2009) emphasises the circumpolar location of a point bar trace assemblage he describes.

OBSERVATIONS

Trace Assemblage

An abundant and moderately diverse trace suite was observed on an emergent sandbar. The traces were produced in a sandy substrate and exhumed by a one-metre fall in the river water level. The unconsolidated coarse-grained sediment that the traces occur in is compositionally mature, moderately sorted, and exhibits a range in grain size between fine-grained sand and granules. Pebble and cobble lags are locally present downstream of the study site and the substrate grain size also increases in the deeper waters situated towards the centre of the river channel. The sediment contains little apparent visible organic matter. The siliciclastic sands

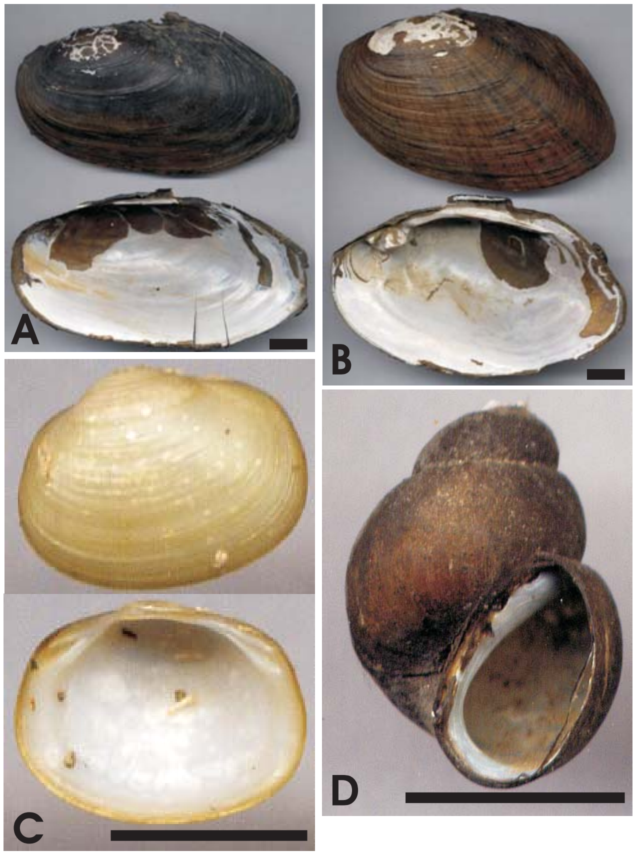

Figure 2.3. Photographs of the molluscan assemblage. **A)** *Anodonta cataracta*. **B)** *Lampsilis radiata*. **C)** Undifferentiated Sphaeriidae. **D)** *Campeloma decisum*. Scale bar for A, B, D = 1 cm; scale bar for C = 5 mm.

are dominated by quartz (80%), but also include phyllite and mafic igneous lithoclasts (10%), feldspars (5%), and micas (5%).

The soft sediment trace assemblage comprised distinct track patterns attributable to three vertebrate genera, in addition to a varied array of trace forms generated by a bivalve and gastropod molluscan assemblage. Hard substrate borings were also noted in unionid shell material, and these included *Oichnus* like borings that are described at greater length in chapters three and microborings documented in chapter four. The invertebrate component of the soft sediment trace assemblage is volumetrically dominant and was formed by a molluscan fauna (Figure 2.3) consisting of the gastropod *Campeloma decisum* (Say), and an abyssate, vagile epifaunal to semi-infaunal bivalve fauna consisting of undifferentiated members of the Sphaeriidae family, together with the unionids *Lampsilis radiata* (Gmelin) and *Anodonta cataracta* (Say) that dominate the assemblage. During emersion, the molluscan trace makers were generally arrested at the end of the traces, either at the sediment surface, or, occasionally, in the case of the bivalves, in shallow, vertical terminal burrows.

The trace assemblage consisted exclusively of modern traces. A pre-omission softground suite (Figure 2.4), comprised varied trace geometries. The range of molluscan generated traces included *Lockeia* like, unionid generated traces of almond shaped morphology. In addition, both the unionid and sphaeriid bivalve fauna were observed to have generated a variety of horizontally aligned furrowed traces. These displayed a range of plan view morphologies, including sinuous meandering, looping and open or tightly coiled forms. In addition to the bivalve generated traces, a multi-lobed trace was generated by the gastropod *Campeloma*. The horizontally aligned molluscan traces were preserved in concave and convex semirelief (epirelief).

Although in two dimensional plan view the horizontally orientated traces bear some morphological resemblance to the ichnogenera *Curvolithus*, *Helminthopsis*, *Gordia*, and *Spirophycus*, as reported in Lawfield and Pickerill (2006), it was not possible to view the full three dimensional morphology of the traces and many of the ichnotaxabases characterising these ichnogenera are absent (e.g. Buatois et al., 1998a for *Curvolithus*; Wetzel and Bromley, 1996; Buatois et al., 1998b and references therein for *Helminthopsis*; Buatois et al., 1998b and references therein for *Gordia*; Häntzschel, 1975 for *Spirophycus*). It would therefore be incorrect

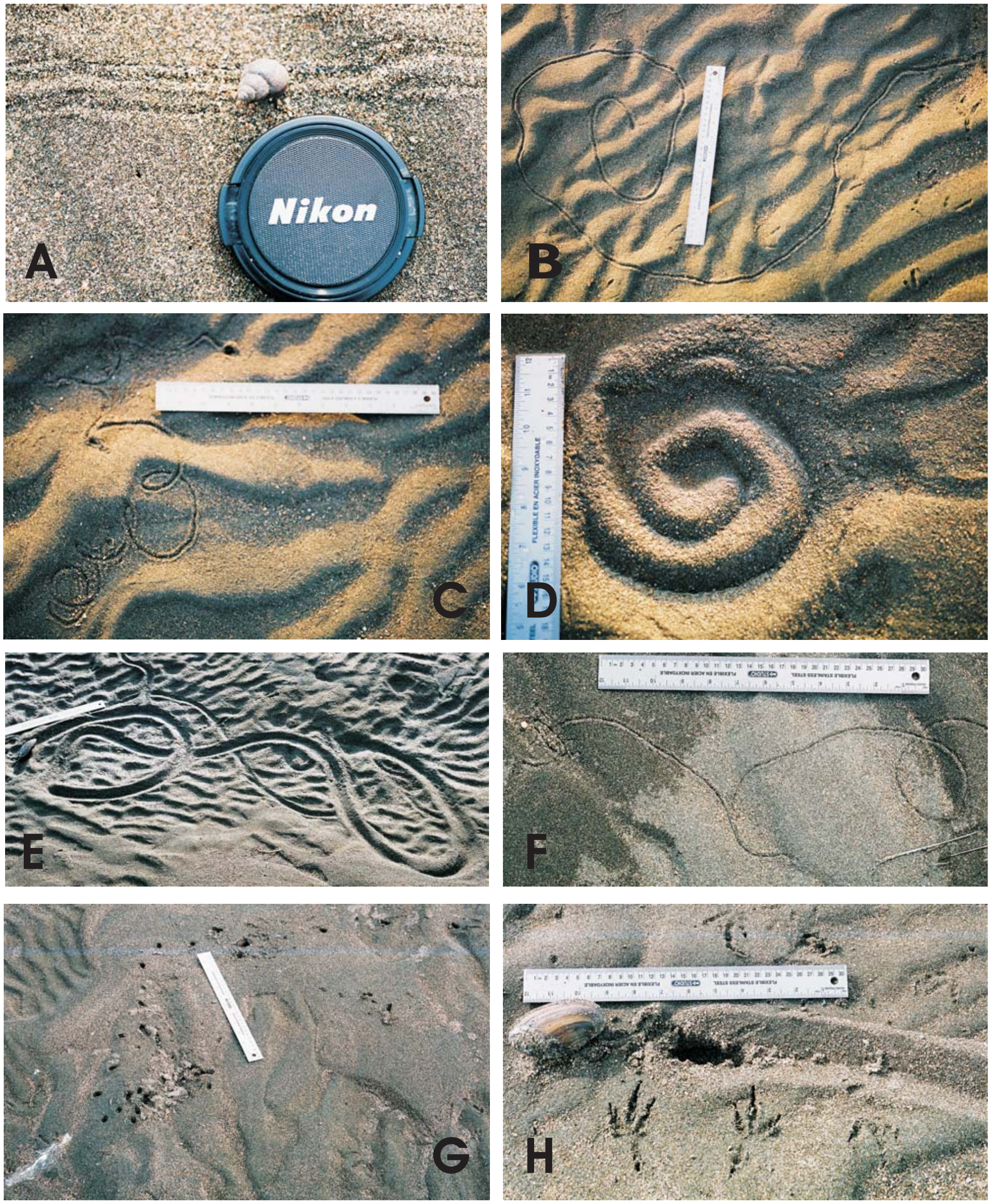

Figure 2.4. Trace assemblage examples. **A)** *Campeloma decisum* producing multi-lobed trace. **B)** Sphaeriidae produced open coiled trace, illustrating dextral coiling and terminal burrowing. **C)** Sphaeriidae produced looping trace, displaying sinistral coiling and terminal burrowing. **D)** Unionid produced tightly coiled trace, exhibiting sinistral coiling and terminal burrowing. **E)** Unionid produced looping trace overlying a meandering trace. **F)** Sphaeriidae produced looping trace, illustrating sinistral coiling and erratic terminal behavior; interpreted to be syn-omission. **G)** Gull-probing trace. **H)** Raven

excavation trace. Except for A, scale bar = 30 cm, fine gradations in cm. lens cap in A = 55mm diameter.

to maintain these assignations and with the exception of the *Lockeia* (James, 1879) and *Oichnus* (e.g. Bromley, 1981; Nielsen and Nielsen, 2001 and see also additional references presented in chapter three) like traces that display all diagnostic criteria, and would be correctly assigned to these ichnogenera if preserved in the rock record, we will refer to the curving molluscan traces in purely descriptive terms, in open nomenclature.

In terms of the ethology of the soft sediment molluscan generated trace assemblage the horizontally oriented traces that represent the majority of the assemblage are grazing/locomotion trails. The *Lockeia* like traces indicate resting behaviour. The simple vertical burrows are associated with attempts by the bivalve fauna to avoid emersion as a result of the rivers falling water level.

In addition to the subaqueous molluscan generated invertebrate trace assemblage, a vertebrate assemblage was present. This included track patterns and probing traces attributable to *Larus* (gull), *Corvus* (raven), and *Mustela* (mink) activity, comprising an omission suite (Figure 2.4) created after the water had receeded.

DISCUSSION

Unionid Ethology

From their Devonian origins (Chamberlain, 2004), unionid bivalves have been an integral part of freshwater ecosystems. They currently dominate fluvial benthic biomass, providing nutrient coupling between the benthic and pelagic realms (Vaughn et al., 2004). Their longevity (frequently >25 years) and slow growth are characteristic of a K-selected strategy, contrasting with the R-selected communities previously associated with the *Scoyenia* ichnofacies (Buatois and Mángano, 1998). Their lifecycle incorporates an obligate ectoparasitic glochidial larval stage, supported by fish hosts that require perennial water bodies (Vaughn and Taylor, 2000).

Vertical burrowing activities are more frequent among endobenthic juveniles as opposed to the dominantly epibenthic summer-season behavior exhibited by adults. Burrowing is carried out as a response to temperature extremes, to avoid desiccation, predation, displacement, or other environmental stresses, and is observed more frequently in soft (silt–clay size) sediments (Amyot and Downing, 1997; Nichols, 1997). Burrowing has been shown to be more rapid and deeper among smaller, thinner-shelled, more motile individuals and species (Nichols, 2002; Saarinen and Taskinen, 2003). Surface-crawling behavior has been attributed to food, oxygen, or spatial competitive environmental stresses, although reproductive, pedal grazing, and trematode parasite induced behavioral abnormalities may all play a part (Saarinen and Taskinen, 2003).

Feeding activities of juvenile unionids and sphaeriids are based upon pedal deposit feeding, a bias that is retained in many adult unionids (80% of consumption), although siphonal suspension feeding takes on an increasing role in some species (20% of consumption; Raikow and Hamilton, 2001). Different habitats may be exploited with different feeding strategies among the same species, with bacteria comprising an increasing food component in shaded, turbid, or low-productivity rivers, where feeding may be observed continuously.

This contrasts with the plankton dominated diet of lake dwelling species, whose feeding activity is diurnal (Vaughn and Hakenkamp, 2001).

Trace Taphonomy

While observations immediately after the emersion event revealed an abundant and relatively diverse trace suite, the preservational potential of the assemblage is poor. The high energy river channel location, with shifting sandbars in an area of net sediment transport, would create a generally harsh taphonomic regime both for the traces and for the associated molluscan assemblage (see further discussion of unionid taphonomy in chapter three). The soft sediment traces described here were seen to post-date wave ripples, and active trace making was observed in shallow (<0.5 m) water. A receding shoreline led to pronounced deterioration in trace definition, with subaqueous reworking observed at the water margin, and saturated, wave agitated sediment forming a softground unable to retain well defined trace margins. Bivalve trace production ceased upon subaerial exposure, with the bivalves' trace making behavior becoming erratic before being replaced by upending that led into vertical burrowing as emersion occurred. Subsequent deterioration of the traces was apparent in those subaerially exposed, with desiccation allowing aeolian transport of sand that removed material from ridges and infilled furrows.

The surface dominated, shallow-tier nature of the trace assemblage contributes to their poor preservational potential, and while “No biogenic structure can be said to have zero preservation potential” (Bromley, 1996), the authors know of no instances of a similar occurrence from the rock record (with Chamberlain et al., 2003, describing a fluvial bivalve trace assemblages characterized by deeper tier activity and Johnston and Hendy, 2005 describing an apparently much lower diversity of morphologically simpler trace forms).

This research supports the well established ichnological principles (Bromley and Fürsich, 1980; Ekdale et al., 1984) that one trace maker may produce a diverse trace assemblage (e.g., unionids generating multiple variations of looping, meandering and coiled furrowed horizontal traces in addition to simple vertical burrows and *Lockeia* like traces. Conversely different organisms may create identical traces (e.g., unionid and sphaeriid generated simple meandering and looping trails).

Fluvial Settings And The *Mermia–Scoyenia* Ichnofacies Models

Miller et al. (2002) documented the delayed colonization of freshwater as opposed to marine substrates throughout the Phanerozoic, additionally documenting the predominantly horizontal and surficial rather than vertical nature of traces in lacustrine settings, which are most frequently bioturbated. The paucity of trace observations in fluvial-channel settings was attributed to erosion. Floodplain settings demonstrated degrees of colonization intermediate between the fluvial and lacustrine end members.

Limitations for the application of the existing terrestrial ichnofacies model have been discussed by Hasiotis (2004). Hasiotis documented a diverse trace assemblage (including bivalve-generated traces) within the Jurassic Morrison Formation of the U.S. Rockies, which ‘‘could occur in any one of the proposed *Scoyenia*, *Termitichnus*, and *Coprinisphaera* ichnofacies based on their broad and ambiguous definitions’’ (Hasiotis, 2004). Hasiotis (2004) emphasized the prominent role played by geographic and temporal variability in hydrologic conditions from intermediate settings between aquatic and non-aquatic.

Variations within the fluvial realm appear to be particularly difficult to encapsulate adequately. Zonneveld et al. (2000) exercised a three fold subdivision of the fluvial system, consisting of: 1) flood-basin or alluvial plain (*Planolites*, rare *Skolithos*, meniscate burrows); 2) crevasse splay (*Arenicolites*, *Skolithos*, vertical shafts, *Camborygyma/Thalassinoides*, *Scoyenia*, *Rusophycus*, *Taenidium*, *Planolites*, *Palaeophycus*); and 3) fluvial channel (vertically oriented burrows with *Camborygma*, cf. *Ophiomorpha*, *Spongeliomorpha*, *Thalassinoides*) divisions. These divisions were distinguished based on their distinctive trace assemblages in conjunction with physical sedimentary structures. In his discussion of the *Scoyenia* ichnofacies, Hasiotis (1997) proposed a four-fold subdivision of the alluvial environment, with channel, levee and proximal floodplain, crevasse splay, and distal floodplain ecological and depositional subenvironments. The channel environment contained a dominantly horizontal-trace assemblage recording the activity of gastropods, nematodes, bivalves, crayfish, crabs, insect larvae, beetles, and oligochaetes. Ethologically, the burrows are constructed for shelter, deposit feeding, and locomotion. Gastropod and clam grazing, feeding, and aestivation burrows are also reported from distal-floodplain subenvironments, together with the permanent benthos sub-environment of lakes.

Buatois and Mángano (2002) discussed floodplain deposits and their implications for continental ichnofacies models, identifying two discrete assemblages. Desiccated floodplain assemblages were attributed to the *Scoyenia* ichnofacies, with a low diversity of invertebrate and higher diversity of vertebrate ichnofossils. The assemblages included backfilled, meniscate, and bilobate traces, with scratch marks and arthropod and tetrapod tracks. Floodplain assemblages represented an impoverished *Mermia* ichnofacies, and contained a low-diversity assemblage of shallow to surficial grazing, locomotion, and dwelling trails and burrows, with poor preservation as a consequence of a water-saturated substrate. Floodplain water bodies are ephemeral and unstable, and therefore require more rapid colonization than permanent lakes; these ichnofacies thus reflect environmental factors rather than purely sedimentary environments, as stressed by Buatois and Mángano (2002) in their discussion of the breadth of environments in which the *Skolithos* and *Cruziana* ichnofacies are encountered. They noted that the *Scoyenia* ichnofacies indicates periodic inundation or exposure, characteristic of lake-margin, floodplain, or wet interdune settings, while ‘‘the *Mermia* ichnofacies indicates permanently subaqueous freshwater conditions’’ (Buatois and Mángano, 2002), conditions that occur in lacustrine basins, fjords, and water bodies formed in floodplain basins. Buatois and Mángano (2004) noted the presence of *Skolithos* ichnofacies in lacustrine settings, specifically within active channel and wave-dominated lake shorelines or lacustrine delta-mouth bar settings. The *Skolithos* ichnofacies assemblage includes both vertical burrows and escape traces. The presence of an assemblage, including rare *Skolithos* traces within a braided-fluvial system, was discussed by Hiscott et al. (1984), whose interpretation invoked marine incursions to explain the presence of *Skolithos*. Buatois and Mángano (2004) described the *Scoyenia* ichnofacies from abandoned or inactive channels and low energy lake margins, where the trace assemblage included a low diversity of meniscate traces. Within floodplain settings, the *Scoyenia* ichnofacies contained abundant arthropod and vertebrate tracks, meniscate traces, ornamented burrows, and bilobate traces with scratch marks. The *Mermia* ichnofacies, found within permanently subaqueous floodplain (impoverished) or lake settings, was comprised of simple grazing trails, locomotion trails, and horizontal dwelling burrows. While emphasizing that ‘‘ichnofacies are not indicators of particular sedimentary environments but reflect sets of environmental factors instead’’ (Buatois and Mángano, 2004, page 315), Buatois and Mángano (2004) also pointed out the importance of sediment water content and substrate consolidation in determining ichnofacies.

Other authors have worked on fluvial ichnological assemblages outside the ichnofacies paradigm. Pryor (1967) described recent point bars with bivalve and gastropod trails migrating downslope, produced when the animals maintained their position relative to a falling shoreline. Trails and burrows exhibiting random orientations were also encountered in flooded back-bar pools. Cretaceous age fossil *Lockeia* and vertical escape traces were reported by Johnston and Hendy (2005) and attributed to the activities of unionid bivalves. Bridge et al. (1986) also undertook work on a bivalve assemblage, with an association being observed between *Archanodon* bivalves and meniscate traces in overbank and channel bar settings of Devonian age. These traces had a predominantly vertical alignment, and were interpreted as escape burrows. The assemblage presented here would appear to be atypical with regard to the complete absence of meniscate burrows. Several authors (e.g., Zonneveld et al., 2000) have recorded vertical crayfish burrows in fluvial-channel deposits. However Gingras et al. (2005) reported that crayfish burrows were conspicuously absent, because they ‘‘are dug deeply only in subaerial settings’’ (Gingras et al., 2005), while Hasiotis (2004) only documented their occurrence in settings subjected to subaerial exposure.

Although it’s preservation potential is evidently remote, should the observed trace suite be preserved, it would include elements transitional between the *Mermia* and *Scoyenia* ichnofacies, as described by Buatois and Mángano (1995, 1998). The pre-omission trace assemblage consists of traces typifying the *Mermia* ichnofacies (e.g. predominantly horizontal traces alongside *Lockeia* like traces), while the post-omission suite vertebrate tracks are exclusively associated with the *Scoyenia* ichnofacies.

The described assemblage was observed in a sandy substrate from a periodically emergent fluvial area, most closely paralleling the environmental interpretations for the modified *Scoyenia* ichnofacies of Buatois and Mángano (1995). However, if the assemblage were to be encountered in the rock record, the majority of traces would place the assemblage firmly in the *Mermia* ichnofacies, whose environmental interpretation suggests an exclusively low-energy, permanently subaqueous lake environment with fine-grained sedimentation. An impoverished equivalent to the *Mermia* ichnofacies (including both *Helminthopsis* and *Lockeia*) has been described from overfilled overbank deposits, which “tend to dominate in proximal overbank settings and/or temperate and humid settings” (Buatois and Mángano, 2004); however, this setting still implies a permanently subaqueous condition. These observations provide a cautionary note against a perhaps too-rigid application of some ichnofacies models, and stress the importance of supporting trace-fossil-based interpretations with appropriate sedimentological observations (Frey et al., 1984). If sufficient occurrences of this and similar ichnocoenoses were to be observed in the sedimentary record, then the formulation of a fluvial channel ichnofacies may be warranted, as originally proposed in Buatois and Mángano (1995). As it stands the assemblage described here may be taken as a composite assemblage (e.g. Melchor et al., 2012).

Whilst most emphasis within this contribution is placed on the invertebrate component of the trace assemblage, which, because of physiological constraints, has the greatest significance for paleoenvironmental reconstruction (Hasiotis, 2004), it should be noted that vertebrate tracks were prominent in the original diagnosis of the *Scoyenia* ichnofacies (Seilacher, 1967). Structures produced by aquatic or semi-aquatic terrestrial vertebrates have been reported throughout the preserved sedimentological record (e.g., Miocene *Diamonelix* burrows attributed to *Palaeocastor*; Martin and Bennett, 1977). Although their utility and applicability have been called into question (see Hunt and Lucas, 2007; Melchor et al., 2012 and references therein, for recent reviews), a number of vertebrate ichnofacies and ichnocoenoses have been proposed for environmental settings that overlap the environments with which the *Scoyenia* and *Mermia* ichnofacies are associated. These include the shore-bird ichnofacies (Lockley et al., 1994), subsequently amended to the *Avipeda* ichnocoenose (Hunt and Lucas, 2007). This ichnocoenose, together with mammal tracks and other components, forms part of the amended *Grallator* ichnofacies that is found in lacustrine margin environments (Hunt and Lucas, 2007). The vertebrate traces described herein might be ascribed to an impoverished example of the amended *Grallator* ichnofacies.

SUMMARY

1) A modern freshwater river channel trace assemblage shows trace forms that if preserved in the rock record would represent an assemblage transitional between the published *Scoyenia* and *Mermia* ichnofacies. The study site from the Saint John River, Fredericton, New Brunswick, Canada, is dominated by elements traditionally assigned to the *Mermia* ichnofacies, with the presence of multiple variations of meandering, looping and coiling horizontally aligned furrowed traces, as well as *Lockeia* like traces.

2) Environmental characteristics are, however, more typical of the *Scoyenia* ichnofacies, with an emersion event providing conditions favorable to viewing traces preserved in a sand softground substrate.

3) Observation of in situ trace-making behavior allowed traces to be attributed to their progenitors, which include unionid and sphaeriid bivalves.

4) An omission assemblage of vertebrate tracks was also present, comprising gull, raven, and mink. In addition, *Oichnus* like borings were observed in some unionid shells.

5) The shallow-tier trace assemblage created in a high energy river channel may be expected to have a poor preservational potential, with loss of trace definition observed at the water margin during emersion and subsequent deterioration by aeolian sediment transport.

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CHAPTER 3

OBSERVATIONS ON THE TAPHONOMY OF A RECENT FRESHWATER UNIONID BIVALVE ASSEMBLAGE, INCLUDING ASSOCIATED *OICHNUS* LIKE PITTING STRUCTURES[1]

INTRODUCTION

Over the last few decades modern day analogue based taphonomic studies have become a well established and prolific field of research. As a result, the processes that operate on marine bivalve faunas and the factors relevant to their preservation or loss to the fossil record are now well documented (e.g. see reviews in Cadée, 1991 and Allison and Bottjer, 2011). Despite this, taphonomic study of the freshwater realm remains, if no longer neglected, then at least comparatively underrepresented in both the biological and geological literatures. Although unionid bivalves have formed an integral part of many freshwater ecosystems since at least the Triassic (Gray, 1988; Watters, 2001; Strayer, 2008; Cummings and Graf, 2010 and references therein) and perhaps from the Devonian onwards (e.g. Cadée, 1991; Chamberlain, 2004; Chamberlain et al., 2004), the taphonomy of both fossil and modern examples has received relatively modest attention, (e.g. Hanley and Flores, 1987; Hinch and Green, 1988; Briggs et al., 1990; Cummins, 1994; Good, 2004; Kotzian and Samões, 2006; Newell et al.,

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[1] *A version of this paper has been submitted for publication. Lawfield, Gingras, Pemberton and Pickerill. Palaeogeography, Palaeoclimatology, Palaeoecology.*

2007; Strayer and Malcolm, 2007; Burton-Kelly, 2008; Nielsen et al., 2008) and where such studies have been undertaken, observations are often focused at the community level rather than addressing taphonomic signatures associated with individual organisms. The documented fluvial and lacustrine localities where these published studies were conducted display a wide range of faunas, sedimentary, hydrodynamic and climatic conditions, as well as variations in the local chemical environment, both during and after deposition. As a consequence of this variability, the associated preservational styles differ widely. One notable feature that has been reported from several assemblages is the presence of pitting and hole features, occurring at various scales (e.g. Cummins, 1994; Nielsen and Nielsen, 2001; Kotzian and Samões, 2006; Lawfield and Pickerill, 2006). These features often display morphologies similar to the boring trace fossil ichnogenus *Oichnus*. They have also been reported to occur in non unionid freshwater mollusc assemblages (Hagan et al., 1998). This paper seeks to provide a detailed description of these pitting and hole features (*sensu* Nielsen and Nielsen, 2001), as encountered in unionid bivalves occupying a cool climate, lotic setting, documenting their development, setting them in the wider context of observed progressive taphonomic shell degradation and proposing mechanisms for their formation.

STUDY AREA

Observations and sampling for this study were undertaken within a dam controlled reach of the Saint John River, located between Fredericton and the Mactaquac Dam that occurs 13 km upstream, to the west of the study site, in New Brunswick, Canada (45°58′ N, 066°42′ W) (Figure 3.1). Although the Saint John River has a single, low sinuosity channel along the majority of it’s course, the section of river

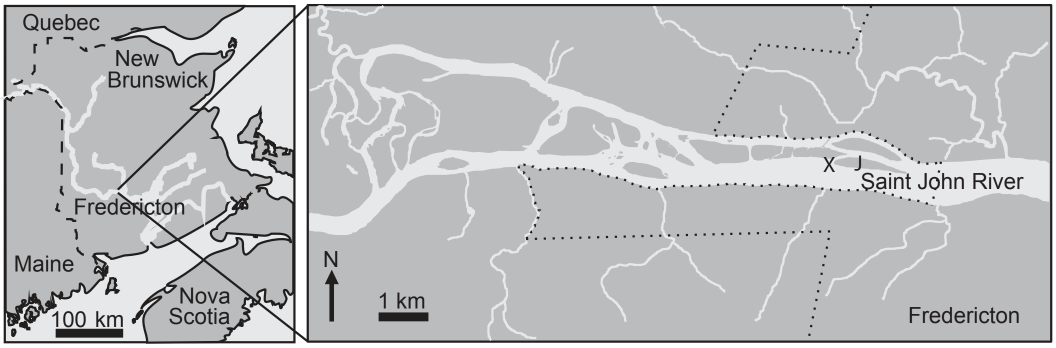

Figure 3.1. Location map depicting the study site (X) at Jewett Island (J) in the locally anastomosing plan view, low (0.03%) gradient Saint John River, Fredericton, New Brunswick, Atlantic Canada (45°58′N, 066°42′W). The Mactaquac Dam forms an ecological barrier, sediment trap and tidal barrier two kilometres west of the inset figured area. Dotted line = Fredericton city limits. After Lawfield and Pickerill, 2006.

where we conducted our study displays an anastomosing aspect in plan view, with approximately 700 m total width of water being locally divided between either two or three channels that are separated by several small islands. Throughout the anastomosing plan section of the river, the largest channel retains a broadly uniform width of approximately 500 m. The river locally possesses a gradient of 0.03% and has a current of moderate speed, with a maximum velocity of approximately 1.5 m/s at the centre of the channel and being of lower velocity towards the banks. Further details of the hydrology are presented in chapter two.

As a result of its location the river is subject to a humid continental climate, with precipitation being evenly distributed throughout the year (Cunjak and Newbury, 2005). The relatively high latitude of the study site results in dramatic seasonal temperature fluctuations, with an almost thirty degree range in daily mean air temperature between -9.3 °C in January and 19.2 °C for July (Wikipedia, 2006). This temperature range results in the development of a thick ice cover over the river during the winter, as well as contributing to significant seasonal variation in the rivers water level, which peaks after the April-May snow melt. Further details of the climate are found in Lawfield and Pickerill (2006) and chapter two. Observations for our study were conducted in September, following a period of low rainfall. Our study focuses upon an assemblage of both live and dead molluscs that is dominated by unionid bivalve remains revealed by the seasonal low water level. The seasonally induced one metre drop in the rivers water level had resulted in subaerial exposure of a portion of channel immediately beside the river banks, as well as several sandbars (shoals) that adjoin the river islands, which are a prominent feature locally within this section of the river.

Sampling took place on an exposed sandbar at the western end of Jewett Island, and in the adjacent shallow water at the edge of the river channel. At this location (depicted in Figures 3.1 and 3.2), the riverbed is dominated by clastic sediment. This predominantly comprises smooth or ripple marked sand, with an increasing proportion of poorly sorted and chaotically arrayed gravel and cobbles being noted as water depth increases towards the centre of the channel.

As a consequence of its wide width the river is subjected to correspondingly low levels of shading by vegetation. As a result of this, rocks on the riverbed are exposed to sunlight, thus favouring growth of a range of periphyton including diatoms that may be exploited by grazing organisms. There is relatively little vegetation in the channel and similarly, locally, low levels of visible detrital organic material deposited on the clastic sediment dominated riverbed. This is presumably a consequence of some combination of the relatively rapid water flow and other factors that may include restrictions imposed by nutrient availability. As a consequence of the channels width there is also a significant wind exposed fetch

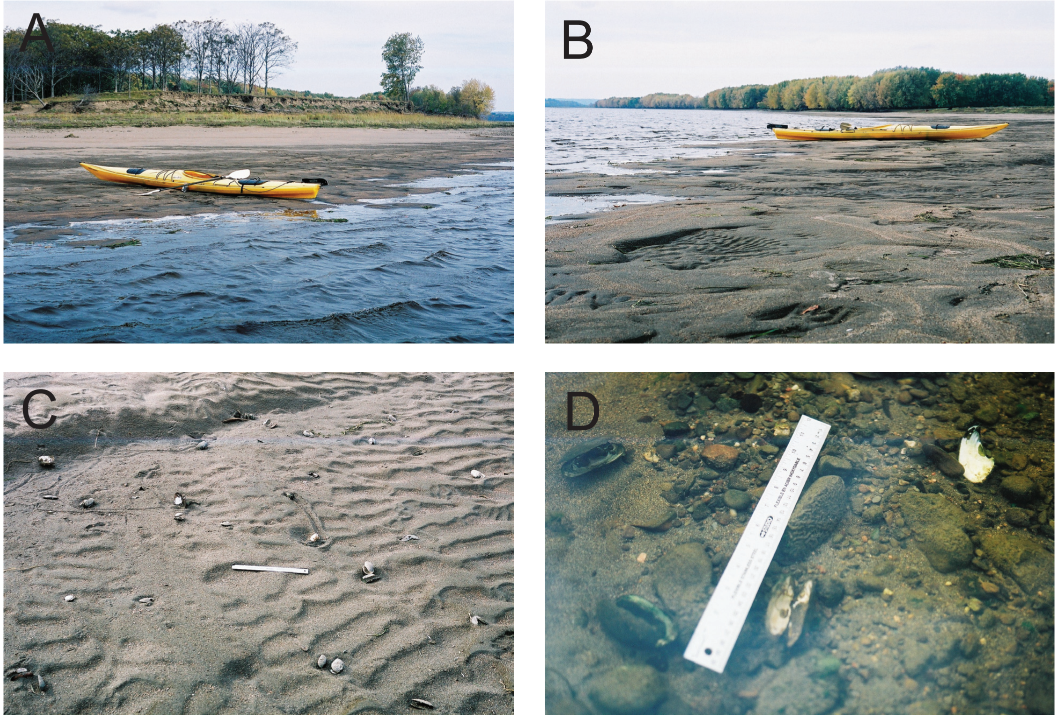

Figure 3.2. The study site comprises a periodically emergent, recently exposed fluvial channel sandbar revealed by a 1 m fall in water level. **A)** Facing northeast towards the adjacent vegetated Pleistocene terrace deposits of Jewett Island. The normal water level is evident from the distinct break in sand colour, with damp recently exposed more organic rich sand present in the foreground. **B)** Facing west, illustrating ripple marked sand and the wide, shallow profile of the river channel. **C)** Exposed ripple marked sand, illustrating the density of the autocthonous biocoenosis and thanatocoenosis. The majority of the bivalve shells are dead and empty, but remain articulated. Live examples are evident at the end of traces described in Lawfield and Pickerill, 2006 (and chapter two). **D)** View of dead, empty, articulated unionid shells in water at the edge of the adjacent main river channel. The 50 cm depth point (1.5 m depth relative to normal water level) marks a transition from a sand substrate to coarser sediment with greater water depth (right). In the deeper water a wide range of grain sizes are represented from sand through to cobbles. Both live and dead unionids were locally absent from deeper waters **(C)**. The kayak is approximately 4.5 m long and the ruler is 30 cm long.

length.

Some parameters for river water chemistry in the St. John River are listed in Curry (Unpublished), cited in Cunjack and Newbury (2005), who report mean values for the waters above Fredericton, through to the rivers source of pH 7.7 (with a range between 7.3 and 8.1) and mean alkalinity of 51.2 mg/L $CaCO_3$ (range 25-102). It should be noted that these measurements refer to the ice-free period.

METHODS

Field Methods

A kayak was used to locate and access an area of Jewett Island together with the adjoining exposed sandbar, which both displayed prominent evidence of molluscan activity, including an obvious assemblage of unionid bivalves and their remains. An accessible stretch of the sandbar and adjacent island riverbank was measured, with a 100 m length being demarcated, within which observations were conducted on all the unionid bivalve specimens observed both on and within the recently subaerially exposed sediment, as well as below the newly established temporary low water level to a water depth of approximately 50 cm (a position equivalent to a water depth of 1.5 m under normal river conditions). This depth point allowed easy and safe access for unobstructed observation of specimens, avoiding any problems associated with the poorer visibility that was a noted feature of the deeper water. This depth also served to demarcate a distinct increase in current velocity, with correspondingly higher grain sizes of riverbed sediment and fewer obvious indications of unionid activity being noted in deeper waters.

All of the accessible bivalve shells occurring on the river bank and sandbar, and in the shallow water, were examined visually. In order to avoid any negative impact on the local bivalve population, live specimens were noted but were left in place. Dead, empty shells in various stages of taphonomic decay were collected and brought back to the lab for further description and analysis.

Lab Methods

Once samples had been returned to the lab they were air dried at room temperature, following which they were disarticulated manually. The identity of the unionid bivalves was confirmed by reference to Clarke (1981). The bivalves were photographed in a light booth, with detailed images of features of interest being obtained with a digital SLR camera with macro lens. The bivalve shells were then sorted visually, based on the nature and degree of shell deterioration to form a progressive series from almost pristine examples, to heavily degraded individuals.

Specimens in various states of taphonomic decay, but all exhibiting clear evidence of both deterioration to the periostracum and pitting in the umbonal region were selected for scanning electron microscopy (SEM) imaging. Preparation for SEM imaging was undertaken, with samples being cut to size by hand with a hacksaw in order to enable them to fit the SEM stubs. The samples were then mounted to the stubs with epoxy resin, before being sputter coated with gold. Imaging was undertaken at 5.0 kV with a Jeol 6301F Field Emission scanning electron microscope. A specimen of the marine bivalve *Epilucina* bearing distinct *Oichnus* like boring was also prepared and examined for comparison with the freshwater bivalve pitting and hole features.

Binocular light microscopy was also undertaken, but owing to the combination of relatively low contrast together with the extremely high depth of field exhibited by many of the samples, this failed to reveal any structures not better resolved by the use of macro photography and SEM analysis.

RESULTS

Overview Of Assemblage

The majority of the observed unionid bivalve assemblage had been recently subaerially exposed by the falling river water level (which reached and retained a position one metre below it’s usual level, for a period of several days). Within this subaerially exposed area, live unionid individuals occurred in a tightly closed shell state. The entire molluscan assemblage was largely undisturbed, with unionids often being encountered either emplaced in resting traces or having been arrested at the end of active movement traces by the falling water level (e.g. Figure 3.2). This soft sediment trace assemblage is described in greater detail in Lawfield and Pickerill (2006), where other non-unionid elements of the molluscan assemblage are also noted. A small minority of the live unionid shells had been disturbed, being subjected to attempted avian predation, but they had resisted this with no apparent damage to their exterior shell surfaces. Below the new water level, live individuals were observed during active locomotion. Dead, empty shells were noted both above and below the water level. Occurrences of the dead, empty shells were interspersed with the live assemblage and the examples observed in the study area showed little or no obvious evidence of post-mortem transportation. Three species of unionid bivalve were noted (Figure 3.3), with

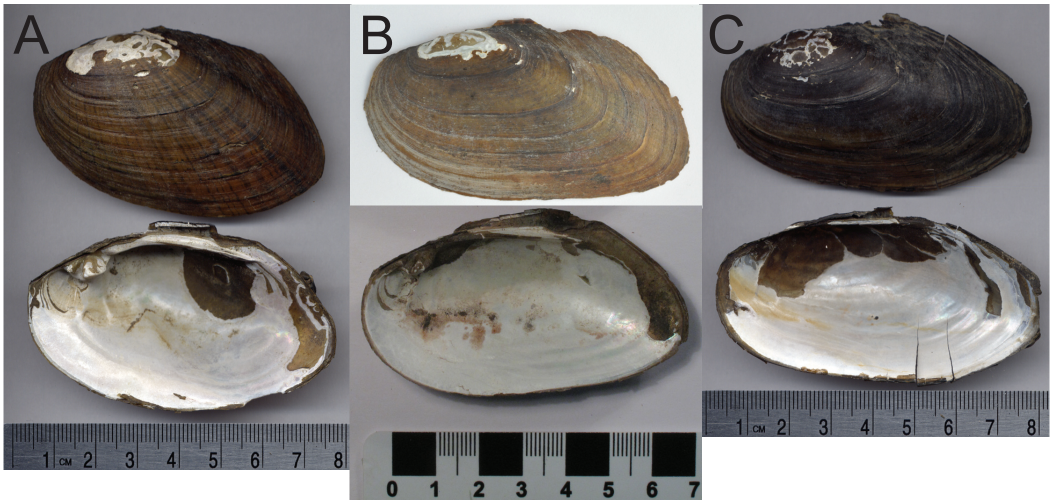

Figure 3.3. The unionid bivalve assemblage includes *Lampsilis radiata* **(A)**, *Elliptio complanata* **(B)** and *Anodonta implicata* **(C)**. *Lampsilis* and *Elliptio* are more robust than *Anodonta* and are more prevalent in the assemblage. Collected *Lampsilis* and *Elliptio* samples display a great range of taphonomic variation and thus form the focus of the present study. Note the prevalence of umbonal etching and decay in all specimens, even on otherwise externally near pristine shell material. The fracturing observed in C occurred as a consequence of the drying of organic shell layers in the thin *Anodonta* shell following its collection. Scale bars in cm.

both live and dead examples of each being represented. They included abundant *Elliptio complanata*, common *Lampsilis radiata radiata* and rare *Anodonta implicata*. The small number of observed examples of the latter species may be attributed both to their forming a smaller proportion of the live molluscan fauna, as well as to the much more rapid destruction of their fragile comparatively thinner shells following death. Due to the small number of *Anodonta* samples collected, the taphonomic observations presented here are illustrated with examples from *Lampsilis* and *Elliptio*, both of which display apparently identical taphonomic signatures.

Examination of the live unionid individuals generally revealed little deterioration across the majority of the exterior shell surfaces, the notable exception being in the umbonal region where some degree of degradation to the periostracum and underlying aragonitic shell layers was universally evident in all examined specimens (See figure 3.4 for a diagram illustrating the shell layers which are described in detail in the discussion). A far greater range of deterioration was noted in the dead shell material. In all instances that we encountered, the dead shells were empty and had apparently been so for some time, not containing any remains of soft tissue and often displaying similar patterns of shell degradation across both their outer and inner surfaces. The dead shells were all found in an open state and almost universally remained articulated, with the ligaments remaining intact even in examples where taphonomic degradation had progressed to an advanced degree, to the point where there were substantial missing portions of shell. Representative examples illustrating the progessive series of taphonomic decay are depicted in figure 3.5, which displays the spatial distribution of most intense shell damage, as well as highlighting those regions of the shell that prove more resistant to the taphonomic processes operating in the Saint John River.

It is worth emphasising that the patterns of shell decay generally showed a remarkable degree of symmetry, with very similar damage being exhibited simultaneously affecting identical regions of both the left and right valves.

Macroscopic External Shell Damage

All the specimens in our study, both live and dead, displayed some degree of decay in the umbonal region (Figure 3.4 and 3.7A). In the more heavily decayed individuals, degradation of the shell exterior appears to have occurred by a process of progressive decay expanding outward laterally from the umbo across the exterior shell surface. Locally, some isolated points not immediately adjacent

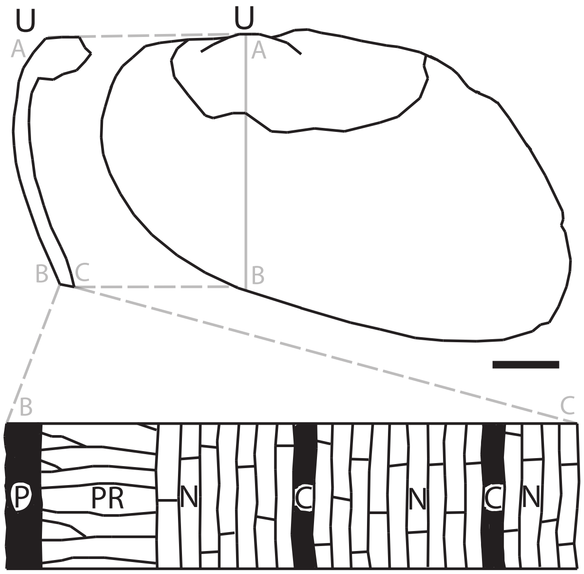

Figure 3.4. Cross sectional diagram illustrating the relative positions and orientations of each of the layers within a unionid bivalve shell. **Top left)** Apical plane cross sectional view. **Top right)** Lateral view. Pale grey line A-B indicates line of section. U = umbo. Line surrounding the umbonal region, in the top right figure demarcates the umbonal area affected by loss of periostracum, with predominantly intact periostracum occurring outside this area. Scale bar for upper images = 1cm. **Bottom)** Cross section B-C. Black regions = organic proteinaceous conchiolin. White areas = crystalline aragonite. P = proteinaceous conchiolin periostracum. PR = prismatic aragonite layer. N = nacreous aragonitic layers. C = conchiolin. The lower cross sectional view is representative and doesn’t illustrate the multitude of nacre layers, which are typically an order of magnitude more prolific than depicted here. Lower cross sectional view not to scale.

to the umbonal region are also observed in a decayed state. These isolated areas of external shell damage are generally associated with regions where the periostracum has clearly been punctured or torn. It is apparent that the periostracum exercises a strong protective influence in preventing or delaying damage to the underlying shell. Once the periostracum has been initially breached, resistant fragments of periostracum may remain in place, serving to obscure

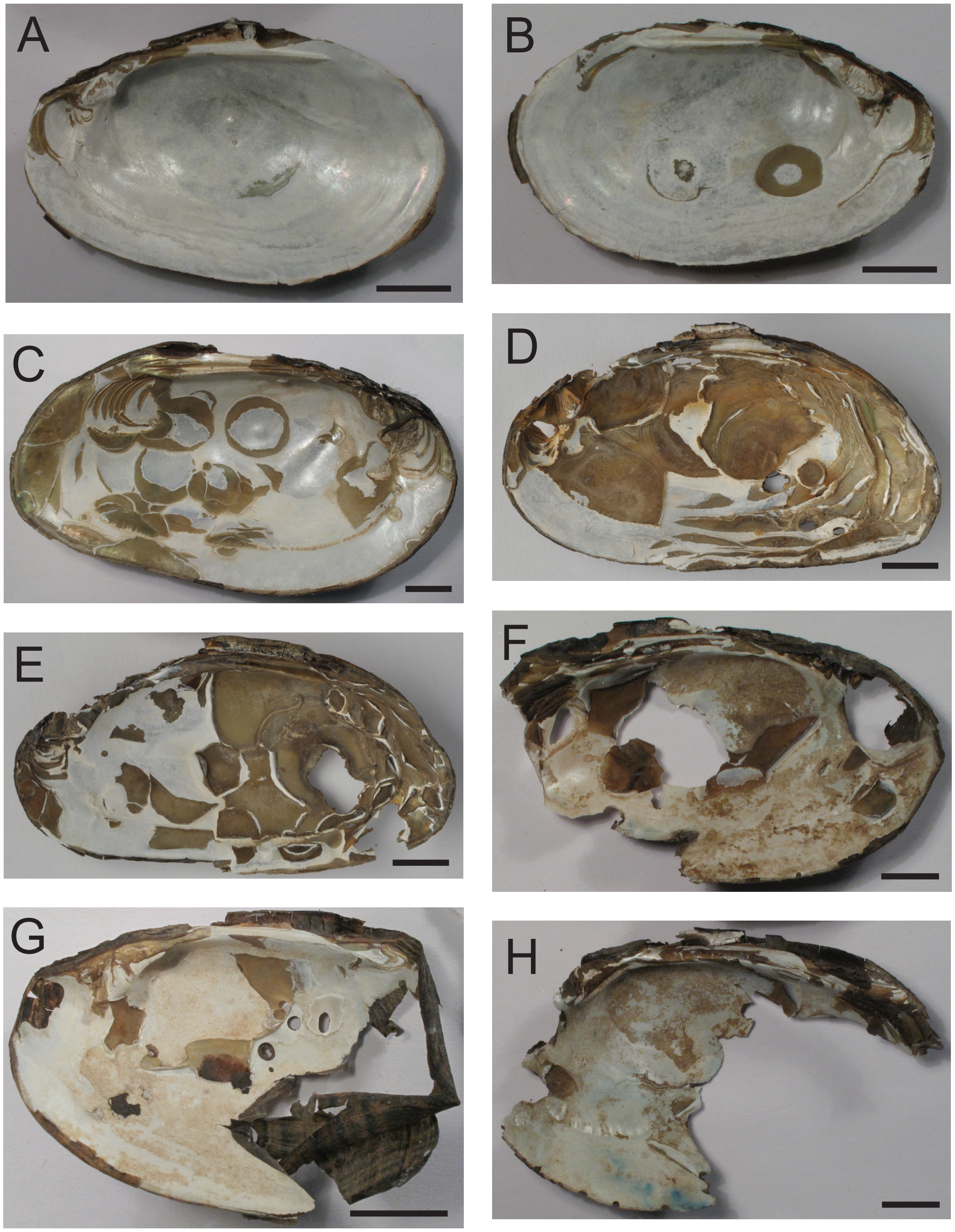

Figure 3.5. Photographs depicting the progressive taphonomic degradation of the interior shell material from near pristine **(A)**, through slightly **(B)**, moderately **(C)** and heavily **(D-E)** to substantially degraded **(F-H)**. The interior of the shell reveals a more progressive sequence of decay than the exterior (e.g. Figure 3.6A). The posterior region of the shell is thinner and is thus seen to be most prone to degradation, which is observed to affect the aragonitic portion of the shell more readily than the organic layers. The periostracum frequently maintains the structural form of otherwise heavily degraded shells. The

presence of the protective periostracum results in preservation of the shell exterior until a point where catastrophic failure occurs once the supportive underlying internal aragonite structure is removed **(e.g. G)**. Over time, a progressive reduction in shell thickness is seen to occur, resulting in exposure of organic conchiolin layers that occur within the nacreous portion of the shell. These conchiolin layers temporarily delay the decay process. Eventually, localised penetration of the entire shell thickness takes place **(D)**, followed by expansion of the initial holes **(E-F)**, finally leading to loss of the anterior dorsal portions of the shell **(G-H)**. All scale bars are 1 cm.

underlying regions of damaged aragonitic shell from view (partly visible in figure 3.5G, figure 3.6A and B and figure 3.7A). Once the periostracum is removed it exposes the underlying prismatic and nacreous shell layers to the environment, allowing decay to take place. This initially results in the formation of pit features, which expand both vertically and laterally. The ongoing development of the pit features results in the removal of aragonitic shell material and the undermining of the periostracum layer. Remnants of periostracum may remain in place as unsupported flaps. Continued downward erosion of the nacreous layers appears to be inhibited by the presence of layers of organic conchiolin interspersed within the nacreous portion of the shell. Once these organic layers are reached, the decay of the shell then proceeds primarily in a lateral direction (Figure 3.6A and B, figure 3.7A, figure 3.8A and C). Eventually, the newly exposed organic layer is finally penetrated and from that point the process repeats itself once the next underlying organic layer is reached. In this manner, a series of stepped terraces may be formed (e.g. Figure 3.7A). In some individual specimens, deposits of silt and very fine sand sediment are found in the pit and hole features that are described in detail in “pitting and hole structures” results section. These sediment deposits are found occurring in both open pits across the aragonitic portion of the shell and also trapped beneath flaps of periostracum, where this layer has been penetrated (Figure 3.6A and B and figure 3.8A and B).

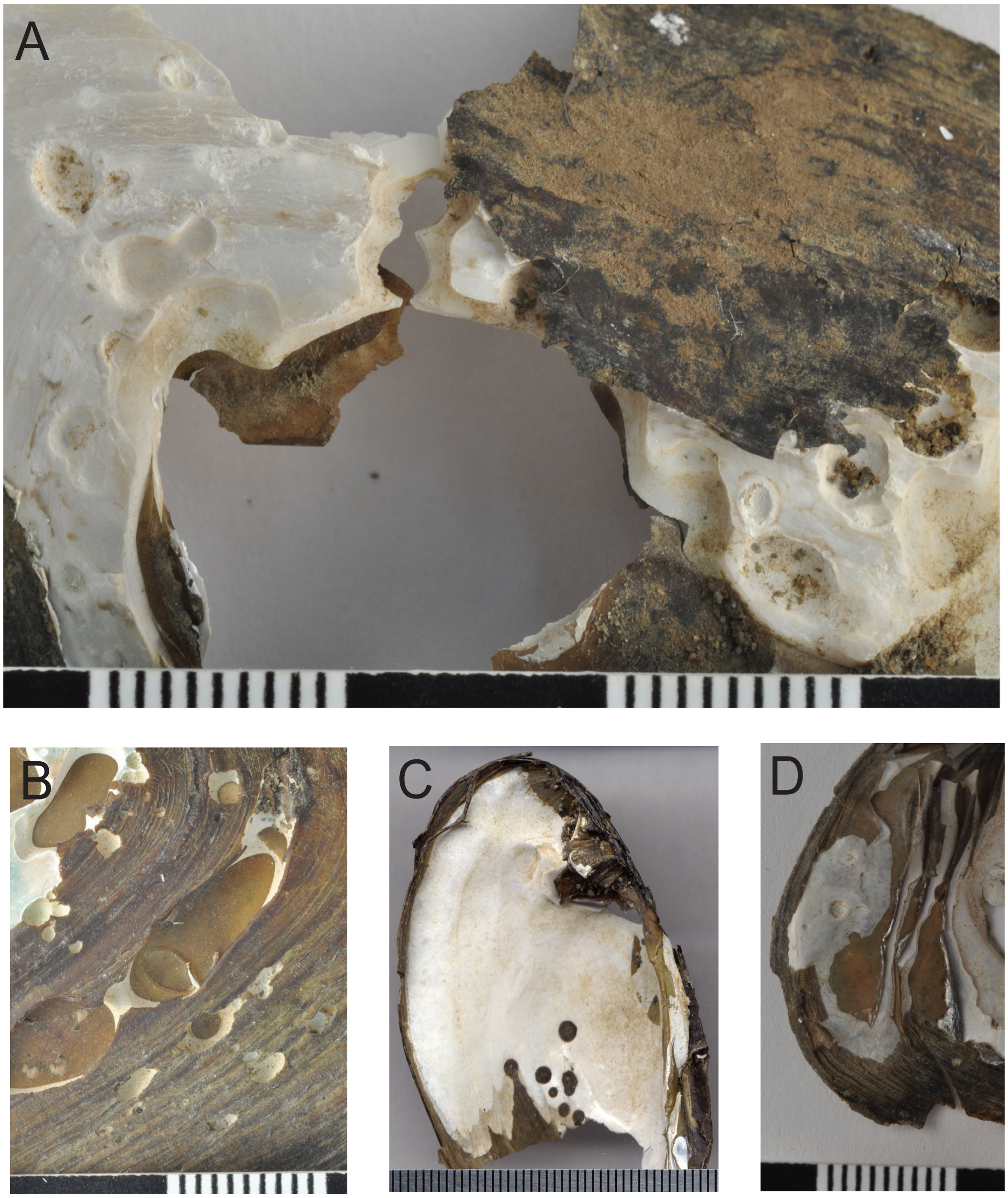

Figure 3.6. Pitting features, with morphologies resembling the ichnogenus *Oichnus* are apparent on both the interior and exterior shell surfaces of the dead, empty unionid shells. The features range from more irregularly shaped pits **(A)** to those of nearly perfectly circular form **(see examples in B-D)**. The features are seen to both partially and fully penetrate the crystalline aragonitic shell layers. Generally the features are more abundant on the shell exterior, and occur beneath areas of punctured, torn or completely removed periostracum, on the exposed underlying prismatic and nacreous shell layers **(A and B)**. Occasionally such features are also noted on the interior of the shell **(C and D)**. The pits sometimes preserve accumulations of trapped silt and very fine sand sediment that may play a role in their formation through physical abrasion **(A and B)**. Fine gradations on

scale bars in mm.

Macroscopic Damage To Shell Interiors

Although patterns of shell degradation are visible on both the shell exterior and interior, a more complete and progressive range of decay is observed internally. Where the shell interiors have been exposed to decay by the post mortem removal of soft tissue, they appear to have been subjected to more rapid degradation than the majority of the observed shell exteriors (e.g. Figure 3.3 which illustrates degradation of the shell interior of individuals that display pristine periostracum across their exterior surfaces, except in the umbonal region).

In common with the processes observed on the shell exterior, removal of aragonitic shell material progressed down to the underlying organic conchiolin layers. However in contrast to the decay of the shell exterior, damage to the interior nacreous shell occurs initially by the formation of cuspate arcs near the margins of the shell (Figure 3.5A to C) and sub-circular rings across the centre portion of the shell (e.g. Figure 3.5 B and C). Loss of the aragonite layers subsequently progresses laterally, following the individual underlying organic layers across the width of the shell (e.g. Figures 3.5 C to E). After the overlying nacreous material is removed, tearing, edge lifting and peeling of the underlying organic conchiolin layers takes place (e.g. Figure 3.5 D to F). Once the full thickness of the aragonitic layers, together with their associated organic material have been penetrated, and the periostracum layer reached, this acts as a temporary barrier to full penetration of the shell (e.g. Figure 3.6 C and figure 3.7 D, F and G). Eventually however the unsupported periostracum fails (Figure 3.5 D to H). Once penetration of the full thickness of the shell has takes place and where this

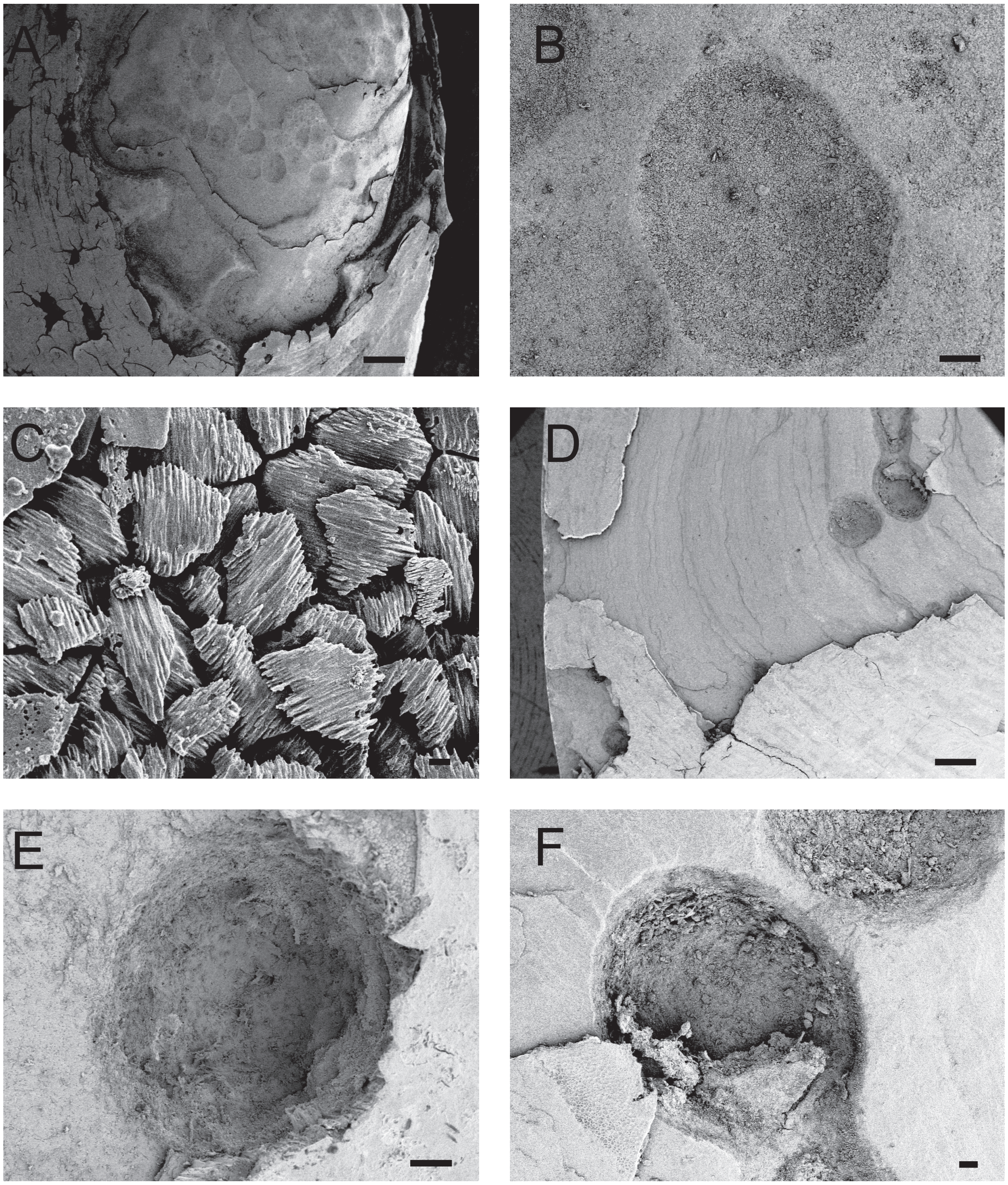

Figure 3.7. SEM images illustrating *Oichnus* like pitting features on the unionid shells exterior surface. **A)** Overview of shell surface, illustrating taphonomic degradation of the exterior periostracum together with the underlying nacreous shell layer with its associated organic conchiolin sheets. The periostracum and interior organic conchiolin layers are punctured and torn, whilst the nacreous layers are heavily pitted. **B and C)** Details of pit illustrated in centre of A. The shallow, irregularly shaped pit feature is marked by the presence of heavily etched aragonite tablets. **D)** Overview of another shell surface, illustrating taphonomic degradation with both torn periostracum and pits in the underlying aragonitic layers. **E)** Detail of pit feature at lower left of D. The irregularly shaped pit is covered in diatoms, extracellular polymeric substances (EPS) and bacteria

similar to those described in Lawfield et al. (In press). **F)** Detail of pit feature at upper right of D. The irregularly shaped pit contains debris including degraded nacre tablets and EPS. The organic conchiolin layer at the lower left is marked by evidence of microboring activity illustrated in Lawfield et al. (In press). Scale bars A,D = 1 mm; B,E,F = 100 μm; C = 1 μm.

has occurred at multiple points across the width of the shell, subsequent loss of the ventral posterior region of the shell may then take place. The region that is lost is generally aligned between the initially established holes (Figures 3.6F through H). The areas most commonly affected by full thickness penetration of the shell or complete loss of distal shell material are in the vicinity of the anterior muscle scar and also in the ventral posterior region of the shell, where they frequently result in the formation of a crudely arcuate, concave or convex edge bordering the lost shell.

Pitting And Hole Structures

An array of pitting and hole features (*sensu* Nielsen and Nielsen, 2001) are present across the shell surfaces (Figure 3.5 to figure 3.8). They are generally most prevalent and well developed across the shell exterior, in particular in the vicinity of the umbo, although rarer occurrences are also noted on the interior of dead empty shells. The features are generally of mm scale and may take the form of either shallow to deep surficial pits, or, more rarely, penetrative holes. The pit and hole features generally display rounded outlines that range from almost perfectly or crudely circular through to ovoid or irregularly curved in shape, the lattermost occurring where several pits of varying sizes, morphologies and orientations appear to be superimposed upon one another. Pit feature with crudely circular outlines appear to be most common. Progressive penetration of all shell layers by the pit and hole features is noted, with the features occurring on regions

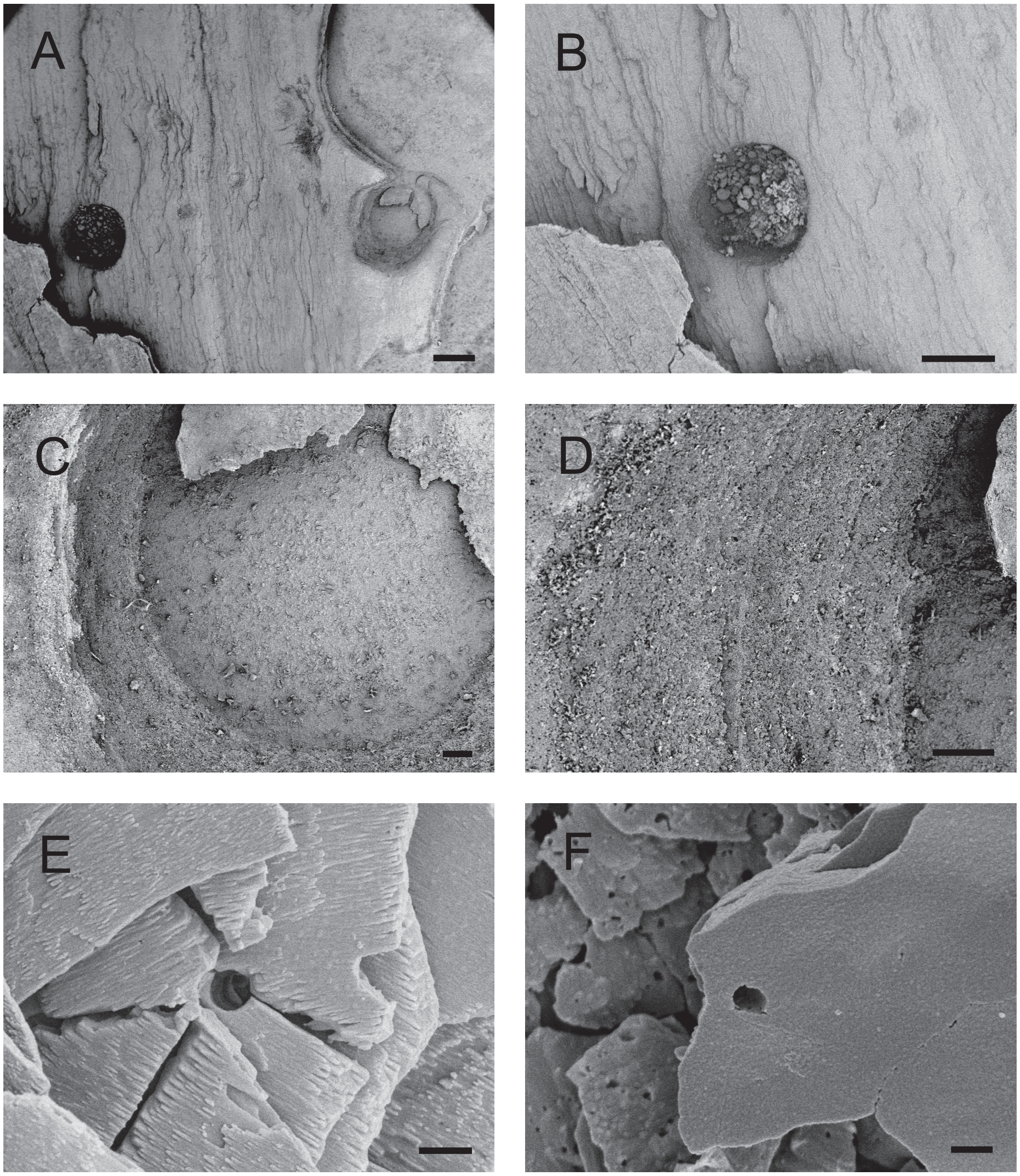

Figure 3.8. SEM images illustrating *Oichnus* like pitting features on the exterior of the unionids shell surface. **A)** Overview of shell surface. Periostracum (lower left) is seen to be separated from a pitted nacreous aragonite shell layer, whilst erosion of the shell locally terminates at an organic conchiolin layer (right). **B)** Detail of pit from lower left of A, illustrating dense concentration of silt and very fine sand grade siliciclastic debris within the main pit. Less deeply eroded pits are visible in upper right of image. **C and D)** Detail of open pit at right of image A. This pit terminates at an organic conchiolin shell layer, which is covered in diatoms, EPS and bacteria (See Lawfield et al. in press). The

steep but irregular wall of this pit feature contrasts with those observed in true predatory origin marine *Oichnus* like borings illustrated in figure 3.9. **E)** Detail of nacreous aragonite tablets in the lamellar shell layer at the edge of the pit seen in C and D. Minor surface etching of the tablets is apparent and the penetrative structure is interpreted as a microboring (e.g. Lawfield et al. in press and chapter four). **F)** Detail of aragonite tablets from the shallow pit features observed in the centre of A and upper right of B. A distinct microboring is seen to penetrate the nacre fragment in the foreground that has been dislodged from the overlying nacreous shell surface. This relatively pristine tablet displays a marked contrast in surface texture to the deeply etched neighbouring nacre tablets within the pit feature. Scale bars A,B = 1 mm; C,D = 100 μm; E,F = 1 μm.

of shell where periostracum has either been completely removed, has been punctured or has been torn. The features are most prominent in the aragonitic shell layers, in particular the nacreous layers. Vertical penetration appears to be inhibited by the organic conchiolin layers that occur in the nacreous portion of the shells. Once the vertical development of the pit and hole features reach these organic layers, they appear to switch to primarily lateral growth, closely following the contour of the organic layers, until these are themselves eventually penetrated. Although much less prevalent, some pit and hole features are present on the shell interior, where they may be confined to the nacreous aragonitic layers (Figure 3.6D), may penetrate the aragonitic layers and terminate against the periostracum (Figure 3.6C), or display a range through to full penetration of the shell thickness (Figure 3.5G). Where the pit features terminate against the periostracum they exhibit sharply defined margins, with morphologies that both overlap and contrast with the generally more irregular margins described previously for examples occurring on the shell exterior (Figure 3.6C).

Comparison of the pit and hole features in our unionid assemblage with similar pit

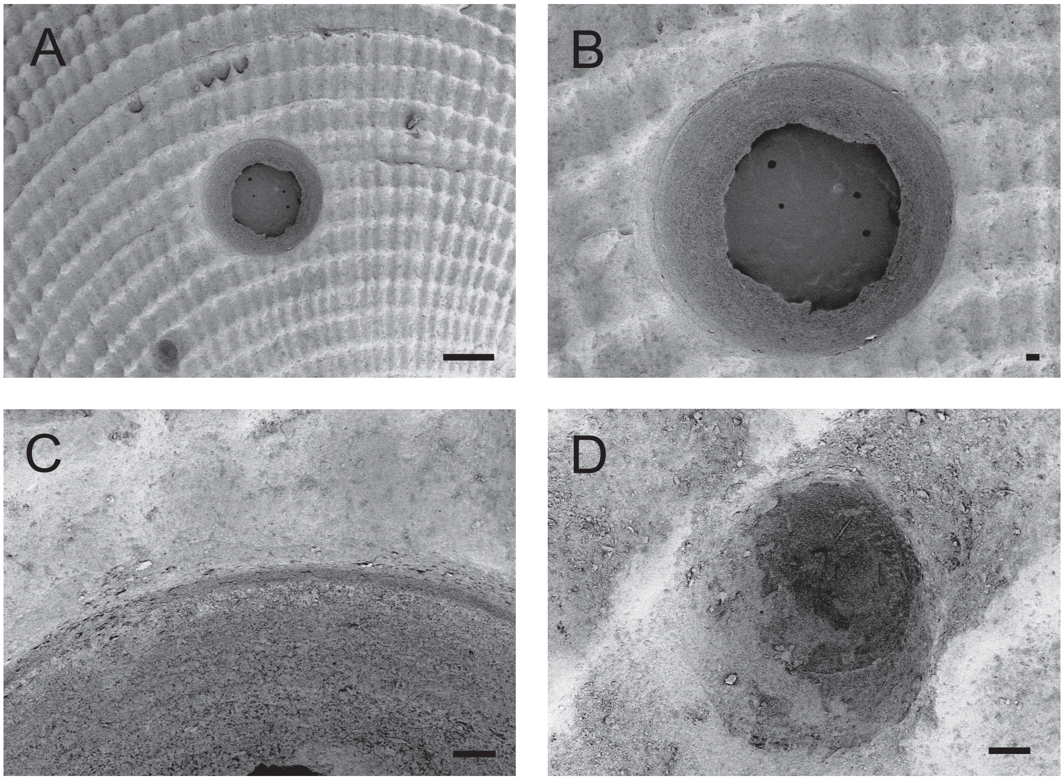

Figure 3.9. SEM images illustrating true predatory marine *Oichnus* like boring in a modern example of the marine bivalve *Epilucina*. Although this bivalve lacks a nacreous shell structure, it is nonetheless possible to compare the pitting features with those observed in unionids (Figure 3.7 and figure 3.8). The sample is a transported specimen, collected from Torrey Pines State Park in Delmar, California and donated by Dr Lindsey Leighton. **A)** Overview of shell surface, illustrating various pitting features as well as a single centrally located penetrative *Oichnus* like boring that exposes the underlying SEM stub. **B and C)** Detailed views of the penetrative *Oichnus* like boring illustrated in A, displaying regularity in both overall form and surface textures. **D)** Detail of non-penetrative pit feature from lower left of A. The overall irregular morphology, rough surface texture and presence of debris are comparable to similar features observed in the unionid specimens illustrated in figures 3.6 through 3.8. Scale bars A = 1 mm; B-D = 100 µm.

features occurring on the shell exterior in a modern example of the marine bivalve *Epilucina* reveals close similarity of the non-penetrative pit features in both taxa, despite differences in the shell structure of the different bivalve taxa. (Figures 3.6 to figure 3.8 and figure 3.9D). There are however significant distinctions between the pit and hole features of both taxa and the true penetrative predatory *Oichnus* like boring feature noted in the *Epilucina* specimen (Figure 3.9). Comparison of the features found in the unionid assemblage with the *Epilucina* hosted *Oichnus* like borings reveals a number of differences between the two, with the unionid features possessing a much more irregular morphology compared to the more precise geometry of the true *Oichnus* like boring. Similarly, the walls of the pit features are much more irregular in the unionid assemblage than the *Epilucina* hosted *Oichnus* like boring that possesses sharply defined surface sculpture.

Microscopic Shell Damage

Observations conducted by SEM imaging allow examination of a number of effects visible only at the micron scale and affecting individual nacre tablets (Figure 3.7C, 3.8E and F). These observations reveal an assemblage of microborings (e.g. Figure 3.8E and F) that are described in detail in Lawfield et al., (In press). Exposed prismatic aragonite is generally noted to be in a pristine state. The nacreous portions of the unionid shells display a wide variation in their degree of decay. Across exposed surfaces, individual nacreous tablets are generally pristine, with sharply defined, angular margins. Within the confines of pit structures, a far greater range is observed, with some tablets showing slight edge rounding and etching (Figure 3.8B). Direct comparison between tablets from the shell exterior and within pit features is possible in figure 3.8C, where a dislodged surface nacre tablet occurs superimposed upon a number of rounded tablets occurring within a pit feature. Heavily etched nacre tablets are only found within pit features (as seen in figure 3.7C).

DISCUSSION

Influence Of Shell Structure On Taphonomic Processes

Our observations indicate a close relationship between both the nature of the decay processes in operation, their extent and intensity, all of which appear to be influenced by and correspond to variations in shell microstructure. Details of the layered shell structure of unionid bivalves and the processes involved in shell formation have been presented and reviewed by a number of authors (e.g. Taylor et al., 1969; Petit, et al., 1980a; Petit et al, 1980b; Petit, 1981; Checa, 2000; Checa and Rodríguez Navaro, 2001; Marie et al., 2007). Describing the layered structure (Figure 3.4), the outer layer is a thin organic periostracum composed of the protein conchiolin and this overlies aragonitic layers that comprise the majority of the shell thickness. A thin prismatic aragonitic layer, with elongate fibrous crystals radiating from spherulith growth centres occurs immediately beneath the periostracum and constitutes approximately 10 % of shell thickness (Checa, 2000; Checa and Rodríguez Navaro, 2001). Beneath the prismatic layer, there is a thick (89% by thickness for *Unio elongatulus*, Checa, 2000) series of nacreous aragonite layers that are interspersed with occasional thin sheets of organic conchiolin that serve to sandwich the poorly to non-aligned nacreous aragonite crystals. The shell interior lying immediately adjacent to the unionids soft tissue is composed of nacreous aragonite.

Wolverton et al., (2010) provide a review of the taphonomic factors significant to the preservation of unionid assemblages in archaeological contexts, unionids having long been exploited by man as a food resource, for tools and ornamentation. They propose that shell forms with higher sphericities and densities promote preservation, in the case of both complete shells and also fragmented shell debris. The density of shells is closely tied to their microstructure, the significant role of which (alongside shell thickness and the presence of organic matrix) for preservation or destruction of shell material is emphasised in Zuschin and Stanton, (2001) and Zuschin et al., (2003). The papers coauthored by Zuschin provide extensive and wide ranging reviews of the properties and parameters that influence shell preservation, addressing in particular those ecological, biostratinomic and diagenetic factors that give rise to shell fragmentation. As noted by Cummins (1994), in comparison with other bivalve taxa, unionids tend to possess thick, robust shells, promoting their preservation. Unionid fossils thus form a common component of preserved freshwater clastic sedimentary deposits in North America, (e.g. Henderson, 1935 and Feth, 1964 reviewed in Cummins 1994). Intrinsic factors of the unionid shells that are listed by Cummins as promoting taphonomic degradation include their weak hinges and high organic content. However, both a literature review of preserved examples and our observations derived from our described modern unionid assemblage suggest that these latter properties are likely to be rather more ambiguous in their effect.

Our observations on the prevalence of articulated shells accord with those of Newell et al., (2007) in that unionids possess “a resilient ligament”. They report however that disarticulation occurs with minimal transport, ultimately giving rise to a predominantly disarticulated assemblage in their study. In the context of articulation, unionid assemblages preserved in the rock record display a somewhat perplexing range of preservation, with Good, (1989) finding that Triassic lacustrine rocks generally contained recrystallised, articulated examples, whilst with the exception of one locality that contained apparently *in situ* examples, river channel assemblages were disarticulated. Disarticulation was also reported for transported crevasse splay assemblages. Good (2004) reports generally similar articulation trends, with silica replaced, primarily disarticulated unionids within a transported river channel assemblage of Jurassic age, whilst floodplain pond deposits preserved articulated specimens. In contrast to the assemblage reported in Good, (1989) crevasse splay deposits described in Good (2004) were articulated. Kues (1985) however reported articulation trends opposite to those described in Goods publications, with an articulated fluvial assemblage and disarticulated lacustrine examples, with recrystallisation in the latter proving finer, enabling better resolution of surface details.

Commenting in her 1997 review of periostracum, Harper notes that "in most recent taxa it seldom lasts intact throughout the life span of the mollusc, let alone surviving taphonomic processes." The vast majority of published studies of fossil unionids accord with Harper (1997) and support our observation that periostracum is most commonly found in a degraded state, across at least part of the shell exterior. Some publications do however report preserved unionid periostracum, with Marshall (1928) describing a Peruvian fossilised Mutilinae specimen of unspecified age exhibiting preservation of both periostracum, prismatic and nacreous shell layers. In a study on Triassic bivalves, Wanner (1921), also reports a "layer about the thickness of writing paper, covering the shell like a periostracum (broken away in places)". The layer is however also noted as being calcareous, suggesting that it may be unrelated to organic periostracum. In all published studies that examine examples from multiple localities, marked variation is noted between the extent of damage to shell periostracum observed at different sites. Once the periostracum has been either removed or penetrated, the underlying aragonitic shell is then exposed and subjected to decay processes. A study by Phillipon and Plaziat (1975) described freshwater dissolution occurring beneath holes in shell periostracum. Kidwell and Bosence (1991) however provide a cautionary note in the interpretation of features that are believed to be the product of dissolution, suggesting that they may in fact be attributed to other mechanisms when subjected to rigorous examination by SEM.

The effect of organic material on the rate of shell dissolution has been discussed by Glover and Kidwell (1993) who note that it may either retard dissolution by creating a physical barrier from the surrounding water, or aid it by providing a growth point for acid secreting microorganisms. The consensus view appears to be that in the case of unionid bivalves, both the internal conchiolin layers and the external periostracum act to retard dissolution (Tevesz and Carter, 1980, Kat, 1983). This trend is supported with observations undertaken on other freshwater bivalves by Isaji (1993). Observations on our own assemblage are certainly in accord with the view that these layers serve to retard dissolution from occurring by abiogenic means, however we also note an apparent correlation between areas of exposed conchiolin sheets within the nacreous portion of the shell and both microborings, as well as the development of sheets of extracellular polymeric substances (EPS) and associated microorganisms (e.g. Lawfield et al., in press).

The prevalence and marked degree of damage to the exterior of unionid bivalve shells, in particular in the umbonal region has been noted by several authors, with Beauchamp (1887) reporting individuals of *Anodonta fragilis* “often worn entirely through the beaks”. Coker et al., (1919) report that “in some streams scarcely a single example can be found with the beaks preserved”. Degradation of the periostracum in older shell regions is also noted by Boyer, (1969) and Hinch and Green (1988) who write “it is often missing from the umbonal region” along with “underlying shell layers”. They account for this by noting that “the umbo is the oldest and presumably weakest portion of the periostracum". Erosion is also noted by Neves and Moyer (1988), who observed a direct correlation between the age of the specimens and the degree of erosion by dissolution, with the area of and around the umbo being most profoundly affected. They also noted pitting of the umbo in older specimens. Roper and Hickey (1994) present a study examining shells of live lake and river unionids that demonstrate two distinctive patterns of erosion, with "periostracum completely missing in a patch originating at the umbo" as the most commonly encountered taphonomic signature, whilst at one sampling location this degradation was extended "often in concentric rings coinciding with some growth bands". Helama and Valovirta, (2007) in describing a fluvial unionid population of high latitude *Margaritifera margaritifera* noted that "even in the smallest specimens the umbo was at least partly lost as a result of corrosion." They suggested that "The progress of shell corrosion decelerated the larger area the corrosion reaches" [*sic*], attributing this trend "to increasing thickness of the outer shell layer". In their discussion of predatory muskrat damage to unionid and more specifically *Margaritifera* shells, Zahner-Meike and Hanson, (2001) note that freshets can move the bivalves, resulting in "erosion of the umbo that is typical of the species". However, although entrainment of the bivalves themselves is certainly possible, we believe that this mechanism is unable to account for the relatively isolated regions of shell damage that are generally observed.

Overview Of Pit And Hole Features And Comparison To *Oichnus* like Borings

Although they have seldom been described in any detail, pitting features and penetrative holes *sensu* Nielsen and Nielsen, 2001 have been previously observed within freshwater settings. Non-unionid molluscan substrates have been noted as a host for putative biogenic *Oichnus* style borings in a lacustrine setting by Hagan et al., (1998). However, the size of the features they report is several orders of magnitude smaller than those in our assemblage, suggesting that a different mechanism is involved in their formation compared to our examples. Surface pitting in older unionid specimens has also been reported by Neves and Moyer (1988), however details of these features and their likely origin are not recorded. In addition, the features herein elaborated on were first reported in Lawfield and Pickerill (2006).

Circular borings in shells and tests are a widely reported phenomenon in the literature relating marine faunal assemblages. Their taxonomy and origin are reviewed in Bromley (2004) and references therein. In reviewing the taxonomy of circular borings, Bromley (2004) and references therein discount the use of the ichnogenus *Tremichnus* (Brett, 1985) which has latterly been considered as a junior synonym for *Oichnus.* Although in their interpretation of Bromley (2004), Donovan and Jagt (2005), suggest that *Oichnus* may itself represent a junior synonym of *Sedilichnus* (Müller, 1977) this usage has not gained wide acceptance and *Oichnus* is therefore retained here. Circular borings in shell substrates were assigned to the ichnogenus *Oichnus* by Bromley (1981), with the initial type species *Oichnus simplex* being subsequently expanded and amended with the addition of several new ichnospecies (e.g. Bromley, 1981; 1993; Nielsen and Nielsen, 2001 and Donovan and Jagt, 2002). *Oichnus* borings may be penetrative in nature, or take the form of a "shallow to deep depression or short, subcylindrical pit" (Bromley, 1981) and their external aperture may be of quite variable form, with "a circular, subcircular, elongate-oval or rhomboid outline" being displayed (Nielsen and Nielsen, 2001). In terms of their ethology, true penetrative *Oichnus* borings are generally ascribed to predatory or parasitic activity, although they may also be produced for attachment (see Bromley, 1981; 2004; Leighton and Aronowsky 2003; Dietl and Kelley, 2006 and Donovan et al., 2006 for summaries of both the taxonomic literature of *Oichnus* and interpretation of causal organisms). In marine assemblages *Oichnus* borings are generally attributed to naticid and muricid gastropods or octopods. They are most commonly reported to occur on molluscs although they may also be encountered on other substrates, e.g. brachiopods (Conway Morris and Bengston, 1994 and Taddei Ruggiero, 1999), crustaceans (Bromley, 1993) echinoderms (Donovan and Jagt, 2002) and even foraminifera (Bromley, 1981 and Nielsen and Nielsen, 2001). Examples produced by gastropod predators, frequently display site selective placement, being sited over thin regions of shell (Bromley, 1981). Single penetrations occurring in the shell region overlying the adductor muscles of bivalve prey, may indicate predation by octopods (Bromley, 1981 and Calvet, 1992).

Boring gastropods employ a variety of mechanisms to penetrate mollusc shells, including abrasion, and chemical attack by acids, chelating agents and proteases. The predatory muricid and naticid gastropods responsible for the creation of many marine *Oichnus* structures are equipped with an accessory boring organ (ABO) (e.g. Carriker, 1961; 1969 and Carriker and Gruber, 1999). In those organisms that possess an ABO, boring is achieved predominantly by a combination of secreted acids and enzymes, aided to a minor extent by physical abrasion from the radula. A number of other marine gastropods are also known to be capable of producing holes that may be of circular morphology, but without the benefit of an ABO (Kabat, 1990 and Ponder and Taylor, 1992). Although no radulation traces were apparent in our assemblage, even where abrasion by the radula is known to occur, such *Radulichnus* traces may be subsequently removed by dissolution (e.g. Carriker, 1969) and it is quite possible that this may have taken place in our assemblage through dissolution by the river water. The marked irregularity of form within the structures we describe is however uncharacteristic of marks associated with predation.

The results of both an experimental study and literature review undertaken by Kelley (2008) suggest that, irrespective of shell morphology and boring placement, the presence of *Oichnus* borings has no effect on the subsequent taphonomic degradation of bored shell material, at least for marine assemblages, where taphonomic degradation is primarily a consequence of physical processes.

Although it cannot be emphatically ruled out, the possibility that our structures represent actively biogenically produced true predatory *Oichnus* like borings is remote. Any explanation for the generation of the pitting features we describe must take account of the presence of pit features below flaps of punctured and torn periostracum. It is hard to envision a mechanism by which a macroscopic organism could access the underlying nacreous aragonite layers without first creating a clean and neat penetration in the periostracum. Given the limited freshwater fauna known to interact with unionid populations and the absence from freshwater environments of any of the three main organisms known to be responsible for the majority of predatory *Oichnus* borings in marine settings, we therefore propose that the features we describe are best assigned to the category of non-predatory *Oichnus* structures. It is however entirely possible that a bacterial agent may be involved in their creation and although bacterial generated pitting of shell substrates does not appear to have been reported in aquatic settings, Smith and Hayward (2010), report bacterial pitting features in an experimental taphonomic study of avian eggshell.

Chemically Induced Taphonomic Processes

The water chemistry of both the river or lake where sampling takes place as well as the underlying groundwater play a significant role in determining the preservation potential of freshwater molluscan assemblages (e.g. Canfield and Raiswell, 1991). Despite unionid shell structure being described by McMahon and Bogan (2001) as being resistant to dissolution, several published studies report carbonate dissolution taking place in freshwater molluscan assemblages (e.g. Coker et al., 1921; Neves and Moyer, 1988; Chamberlain 2004; Chamberlain et al., 2004). A number of sources serve to influence water chemistry, including the bedrock and soil of the catchment area, the nature of the local vegetation, and in the case of present day assemblages, the effects of anthropogenic activity. Discharge is another significant factor, serving to dilute available ions (Giller and Malmqvist, 1998). The chemical properties of most relevance to carbonate preservation include acidity (pH), hardness (dissolved Ca and Mg), HCO_3 alkalinity (concentration of carbonate) and aragonite saturation index (Salminen et al., 2005). Strayer and Malcolm (2007) observed differences in the degree of shell decay at different sampling locations. They attributed this to variations in water chemistry, including Ca, pH, and dissolved inorganic carbon, in addition to water movement. Factors affecting these varied properties of stream water are reviewed in Giller and Malmqvist (1998) and Ander et al., (2006). In some aquatic ecosytems, unionid shell remains may represent a significant Ca sink and reservoir (e.g. Green, 1980 and Strayer and Malcolm, 2007).

There is a close relationship between the vitality and viability of freshwater molluscan populations and the chemistry of their surrounding environment. In addition to their role in reducing the odds of shell preservation, acidic waters may exert an influence upon both physiological processes (Kat, 1982; Pynnönen, 1991 and Mäkelä and Oikari 1992) as well as various ecological effects, ultimately creating a hostile environment unfavourable to molluscan habitation. Acidic waters also restrict the availability of food organisms (Giller and Malmqvist, 1998). These negative effects are however associated with conditions of environmental chemistry that fall significantly outside the ranges reported for our study site, which appears to host a healthy, unstressed unionid population.

Despite possessing pH and alkalinity within a range that is entirely compatible with the possibility of preservation of aragonite, in the absence of a Ca value it is impossible to calculate the aragonite saturation index for the waters of the Saint John River and thus to determine the short term stability of our assemblage. Cummins (1994) reports comparable values of pH and alkalinity to those in our study that would nonetheless result in dissolution. Likewise, details of groundwater chemistry, which would impact the long term preservation potential of our assemblage were unavailable. As noted previously in the “influence of shell structure on taphonomic processes” section of the discussion, the presence of organic layers in the unionids shell structure, in particular the external periostracum have a significant impact on dissolution rates. Direct evidence of dissolution has been observed in our assemblage, generally within pit features where etching of individual nacre tablets is noted (see “microscopic shell damage” results section). Purely chemical dissolution also appears to be a significant factor in loss of aragonitic material from the exposed interiors of dead shells. Dissolution by purely chemical means may have been hastened by the low water temperatures that occur for much of the year and since water chemistry analysis were only available for the ice-free period it is possible that more taphonomically aggressive chemical conditions may prevail during the remainder of the year. As a potential modifier and caveat to the preceding discussion, it is worth remarking at this point on the preservation state of other components of the molluscan assemblage. At this locality, these included the presence of live individuals of the gastropod *Campeloma desisum* with uncorroded early whorls. This state is remarked by Clarke, (1981) as being uncharacteristic except in areas with lime rich waters, with marked corrosion generally being much more typical. Another factor that should be born in mind is the longevity of unionid bivalves, which is typically on the order of decades and may even exceed a century in some species at higher latitudes (e.g. Strayer, 2008 and references therein), during all of this period, the live shell material could be exposed to dissolution processes where the periostracum has previously been damaged or removed. In addition to this, the continuing post-mortem exposure of the shell to the water column, both prior to and following shallow burial would together result in an extended period of exposure of the unionid shells to potential dissolution. Overall, given the observed surface morphology of the exposed nacreous aragonitic portions of the shell, together with the presence of distinctive etching of some aragonite nacre tablets (Figure 3.7C and figure 3.8F), we believe that dissolution by chemical means likely represents a significant component of the taphonomic decay of these shells.

Physically Induced Taphonomic Processes

Several authors have addressed the significance of physical taphonomic processes operating upon freshwater molluscan remains (e.g. Hinch and Green, 1988; Roper and Hickey, 1994; Newell et al., 2007 and Strayer and Malcolm, 2007). Hinch and Green (1988) concluded that, based on observed trends in the degree of taphonomic degradation matching variations in energy levels (reflected by water turbulence and sediment particle size) but not showing a relationship to lake water chemistry (in particular pH and alkalinity) that physical processes are the dominant cause of decay in lake unionid populations. They invoke a combination of abrasion by moving sediment particles, together with the movement of dislodged shells as they contact the substrate of the river bed as being the dominant agents responsible for shell damage. Some indication of the degree of abrasive damage that unionid populations are subjected to can be taken from the fact that in an experimental study conducted by Neves and Moyer (1988), specimens marked with fingernail polish on their shell margin were completely cleared after a three month period due to abrasion with the substrate. In a study of taphonomic processes affecting unionid bivalves in a river setting, Newell et al., (2007) also observed a marked predominance of physically mediated degradation, noting a sequence of decay in which "areas of abrasion evolve into perforations and perforations coalesce and enlarge into fractures". In their assemblage, they observed that disarticulated shells largely remained stationary, being fixed in place in a convex-up orientation, generally with the anterior of the shell facing upstream and the shell being rigidly emplaced at the surface of an "armoured gravel bed" where they were subjected to the abrasive impact of bedload. They also noted a near complete absence of damage to the shell interiors that they describe as pristine, even in individuals with heavily abraded exteriors. In addition damage appeared to be confined to dead shells, with live shells not showing damage in the umbonal region. This contrasts with the universal presence of damaged umbonal shell regions described herein and initially presented in Lawfield and Pickerill (2006).

There are also visible differences in both the appearance and distribution of the degradation patterns evident in the examples figured in Newell et al., (2007) and our assemblage, perhaps as a consequence of the fixed shell positions noted in their study, contrasting with our more freely mobile bivalve assemblage (compare figure 3.2 C and D, this chapter with Newell et al., 2007 figure 6 C and D). In addition to these distinctions, the area of external shell most heavily degraded in our examples is the umbo and the surrounding region, which was not the case in the assemblage described by Newell et al. (2007). The life orientation of the unionid population will obviously have a profound effect upon the degree of exposure of the shell to any abrasive agents, both within the sediment as the bivalves burrow and also in the form of particles mobilised in the water column and impacting the shell. Our observations of both highly variable alignment of live shells located above the surface of the river bed substrate and burrowed shells positioned with their umbonal regions buried below the sediment surface (described in Lawfield et al., 2006), which match patterns observed by Trueman (1968) and those reviewed in Bridge et al., (1986) suggest that the umbonal region of the shell would be largely protected from abrasion, excepting any abrasive damage that might be created directly during burrowing activity. Based on typical orientations of partly buried shells, the lateral dorsal region of live shells should be subject to the greatest abrasive action as a result of entrained sediment particles. Studies in the literature also support this, reporting that except in locations subjected to episodic discharge, the majority of sediment emplaced unionids are found orientated with their siphons (posterior) upstream, (e.g. Zhadin, 1939 cited in Eagar 1978 and Di Maio and Corkum, 1997) and shallow burial of two thirds of each shell (a behaviour interpreted as being undertaken in order to reduce drag and the possibility of dislodgement), with the exposed part being more weathered than the buried portion. Dr Carole Stein (personal communication cited in Eagar, 1974) reports that in rivers with “appreciable” current, unionids burrow to a depth where “only the apertures or the posterior part of the shell (perhaps a quarter of its length)” protrude above the substrate. They may also be found lying on one valve on the substrate surface (Eagar, 1974). All of these observations suggest that where exposed above the sediment surface, the umbonal region would be unlikely to be preferentially subjected to abrasive damage when compared to the rest of the shell surface.

Although there is no conclusive evidence for their having played an active role in the formation of our assemblage, the possibility that physical mechanisms may have contributed to the taphonomic decay we observed is however suggested by the presence of trapped silt and very fine sand sediment in some of the external pitting features and beneath flaps of torn or punctured periostracum. Abrasive damage by entrained sediment impacting exposed shell nacre may have contributed to the formation of the external surface sculpting in the umbonal region of the shell.

Biologically Mediated Taphonomic Processes

Freshwater mollusc assemblages are subjected to the attentions of a diverse array of predatory organisms (see review in Cummings and Graf, 2009). Predators include: amphibians, birds, crayfish, fish, flatworms, mammals and reptiles (Strayer, 2008 and Walker et al., 2001). The predatory activities of most of these organisms however create distinctive patterns of shell comminution (e.g. Elbroch, 2003) that are not matched by the examples of shell damage seen in our assemblage. For adult unionid populations, muskrats together with other mammals such as otter and raccoon (e.g. Strayer, 2008) are thought to represent the principle predators, with muskrat present from both native populations in North America and from introduced examples in Europe (e.g. Bauer, 2001, Zahner-Meike and Hanson, 2001). In the nearshore areas of large waterbodies, the effects of muskrat predation may be sufficiently intense to locally “largely eliminate mussels” e.g. Zahner-Meike and Hanson (2001) and references therein. Preferential predation may take place upon particular size cohorts, the preferred size depending on the unionid prey species. For example, *Pyganodon cataracta* are thin shelled and predation of this species is principally upon larger (>90mm) individuals. In contrast, in the case of thicker shelled species, generally smaller individuals are targeted (Zahner-Meike and Hanson, 2001).

In addition to predators, there are also a wide range of parasitic and symbiotic organisms that may occur in association with unionids, but are not known to cause damage to the shell structure. These are reviewed in Cummings and Graf (2009) and include: bryozoans, copepods, insects, leeches, nematodes, oligochaetes, protozoa, trematodes and unionicolid mites. In addition, Beckett et al., (1996), reported the exploitation of unionid substrates by Bivalvia, Cnidaria, Coleoptera, Diptera, Ephemoptera, Hydracarina, Isopoda, Oligochaeta, Trichoptera and Turbellaria. Microbial mats and unspecified ‘algae’ and ‘aufwuchs’ are also noted in chapter five and references therein.

Numerous organisms are capable of creating penetrative boring structures via either physically or chemically mediated mechanisms, or some combination of the two. The overwhelming majority of studies relating such interactions address examples from marine settings and many of these are listed in Carriker (1961) (and see also our discussion relating to the production of *Oichnus* features, presented in the “overview of pit and hole features and comparison to *Oichnus* like borings” section of the discussion). Several organisms were encountered in association with our unionid assemblage, at least some of which could potentially play a role in damaging the unionids shell surface. The wider molluscan fauna included a number of freshwater gastropods, although all of the examples we encountered in the vicinity of unionids were observed in a free living state, independent from the unionid bivalves. We also noted an array of both microscopic and macroscopic scale epibionts occurring attached to the unionids in our assemblage, including Trichoptera, biofilms and microbial mats (e.g. chapters five and six).

The biology of freshwater gastropods is reviewed in Brown and Lydeard, (2009) and references therein. Availability of calcium is a limiting factor for gastropod populations, although the threshold below which this is significant (5mg/L $CaCO_3$) is exceeded by a significant degree in the St. John River. In terms of obtaining calcium most freshwater gastropods appear to gain this from environmental rather than dietary sources. (e.g. McMahon 1983; Lodge et al 1987; Dalesman and Lukowiak, 2010). The exploitation of shell material as a calcium source has been reported by gastropods inhabiting terrestrial settings (e.g. Cadée, 1999 and Bond and Diamond, 2007), however such behaviour hasn't been previously reported in aquatic gastropods and in any case would presumably be less prevalent in settings where the availability of calcium is not a limiting factor, as is the case at our study site, so would appear to be an unlikely source of shell damage in our assemblage. Schiffbauer et al., (2008) present a number of criteria for the identification of predatory microborings, based principally on the presence of radulation marks. Boring has not been previously reported by freshwater gastropods and they lack an ABO or boring adapted radula, both of which are adaptations associated with marine gastropods that conduct predatory boring behaviour. Although there are no reported instances of predatory boring by gastropods in freshwater settings they may nonetheless make use of a rasping action with their radula as part of their periphyton grazing feeding strategy, which has been reported to occur on both macrophyte and cobble substrates. Some freshwater gastropods are quite selective in their feeding habits, targeting specific fauna for grazing. Although we observed no evidence for interaction between the unionids and other molluscs in our study site (which included gastropods), populations of freshwater gastropods have been noted to graze the surfaces of other individual gastropods of the same or different species (Abbott and Bergey, 2007). Some gastropods also possess radula "adapted to surficially eroding carbonate substrate...to exploit the endolithic microbes as a food source." but these are only known to occur in marine species (Reviewed in Bromley, 2004 and references therein). We see no evidence in our unionid shell samples for the characteristic 100 μm scale *Radulichnus* sculpting that gastropod grazing would create. Detritivory is another commonly adopted freshwater gastropod feeding strategy, although again this is not evinced in our assemblage. Bromley (2004), also reviews reports of an array of etched or abraded "home depression" shell scars on a variety of molluscan shell substrates, that are produced by a range of marine gastropods, but again these have not been recorded in freshwater settings. Although we didn't note any indication of radulation markings or other characteristic sculpting on our unionid specimens, this doesn't confirm their absence, since dissolution and other processes can subsequently degrade the shell surface (as illustrated in the true predatory *Oichnus* like boring in the marine *Epilucina* bivalve shell substrate seen in figure 3.9). The presence of multiple penetrative holes as noted in many of the unionid shells we examined would also be inconsistent with a predatory origin. Given this combination of factors, we therefore consider it reasonable to dismiss gastropods as a potential agent of shell decay at this location.

Trichoptera are observed attached to dead unionid shells in our assemblage and have been encountered elsewhere on live unionids (e.g. see chapter six). However no causal link was observed between Trichoptera and regions of damaged shell and in fact the benign attachment of Trichoptera occurred preferentially on regions of pristine shell.

Unionid shells from our assemblage supported growths of biofilms (comprising a variably proportioned assemblage of bacteria, diatoms, and some combination of cyanobacteria, cyanophytes or fungi, all of which occur together with associated extracellular polymeric substances (EPS)). Details of the biofilms are presented in Lawfield et al., (In press). The biofilms are observed to occur in close association with both microborings and exposed organic conchiolin sheets located within the nacreous aragonitic shell layers. Their presence is particularly common within pit features, but also occurs elsewhere across the exposed nacreous shell. They are therefore evidently associated with damaged regions of shell, although they are unlikely to be solely responsible for producing shell damage, since they are not universally present on exposed nacreous shell and appear to be absent from the periostracum. The significance of microbially mediated shell erosion upon aragonite shells with an organic component is emphasised in Glover and Kidwell (1993). Their study of marine settings records microbial populations more than doubling shell decay rates in comparison to populations exposed to purely chemical dissolution processes. Glover and Kidwell (1993) suggest that the organic component of the shell is exploited as a food resource and its removal subsequently hastens other physical and chemical decay processes (see also “influence of shell structure on taphonomic processes” section of discussion). The patterns of shell erosion we observed are visually similar to the forms illustrated in Harper (1994, figure 5) who depicts non-predatory boring in the aragonitic shells of corbulid bivalves. In particular, the frequent termination of erosion at layers of conchiolin matches well with the patterns we observed in our shell assemblage.

We also observed thick growths of microbial mat on a number of live unionids (e.g. chapter five). Microbial mats may develop upon a preexisting foundation of biofilm. Microbial mats possess a number of properties that could potentially either increase or inhibit shell erosion, as discussed in chapter five, with other examples of unionid associated 'algae' and 'aufwuchs' also being noted in the references included therein.

Review Of Dominant Causal Processes And Overall Preservation Potential Of The Assemblage

Historically taphonomic studies addressing bivalve material have focused primarily on marine assemblages. A range of mechanisms for taphonomic degradation of bivalve shells are presented in studies by Kidwell and Bosence (1991), and Parsons and Brett (1991). Broadly the factors they report as significant fall under the categories of fragmentation, disarticulation, corrasion, dissolution, rounding of the margin, encrustation, and size selection. Flessa and Brown (1983) also describe a variety of mechanisms by which calcareous material degrades, principal amongst them being a dissolution sequence characterised by "development of a chalky texture, thinning of distal margins, surface etching and formation of holes in bivalve muscle scars." Loss of shell lustre and colour were also noted. Significantly they also note that although other factors being equal, aragonite is more prone to dissolution than low magnesium calcite, that the density and surface area to weight ratio of the shell exert a greater control on degradation than does the mineralogy. They also noted that dissolution proceeded from initial destruction of the edge of the shell towards the umbo, which was found to be most resistant to the effects of acid dissolution, observations that corroborate those of Yeo and Risk (1981). Flessa and Brown (1983) also contrast the effects of acid dissolution with those of physical abrasion that frequently results in the opposite taphonomic pattern, with shell damage initiating from the umbonal region. Extrapolating from these results to our assemblage would suggest that the effects we noted on the shell exterior primarily originate via physical processes.

Factors affecting the rapidity of decay of shell material in freshwater settings are reviewed by Strayer and Malcolm (2007). They differentiate between those factors innate to the organism itself, which they term intrinsic factors and those belonging to the environment, which they refer to as extrinsic factors. The intrinsic factors include "shell size, $CaCO_3$ crystal size and mineralogy, chemical composition and structure of organic matter". The extrinsic factors include the water chemistry, water movement and mechanical abrasion. In addition to the aforementioned list, the potential importance of microbially mediated decay processes, particularly in carbonate saturated waters is indicated by Glover and Kidwell, (1993) who contrast the predominant decay processes in waters that approach $CaCO_3$ saturation with those operating in undersaturated waters. In saturated waters, the microbial decay of organic matter was found to be a limiting factor determining the subsequent exposure of $CaCO_3$ crystals to physical (abrasion) and chemical (dissolution) decay processes. For undersaturated waters, chemical dissolution was determined to be the rate limiting process. A comprehensive review of factors of particular significance in the preservation of death assemblages in freshwater settings is also presented in Pip (1988) and references therein. This list is

Turbulence
Shell size
Shell construction
Sorting processes in higher energy environments
Reworking of older deposits containing molluscan shells (from Bajc 1986)
Temporal differences in hydrodynamics (e.g., with differences in bottom conformation or water level)
Differences in sedimentation rate (from Kidwell 1985)
Differences in water chemistry
Oxygen availability
Temperature regimes (which may affect remineralization rates)
Exposure of sediments to freezing in very shallow water
Drying and subsequent rehydration of sediments
Bulk deposition as a result of storm activity
Biased destruction of thin-shelled species in older strata through sediment compaction
Biotic factors, such as vegetation abundance, microbial environment, bioturbation
Episodic differential mortality of different species due to outbreaks of species-specific disease and parasites (giving overrepresentation of stricken species in sediment)
Recycling of benthic shell material by living gastropods in calcium-poor waters
Interspecific differences in organic matter content of the shell

Table 3.1. Taphonomic factors impacting freshwater molluscan death assemblages. Reproduced from a list presented in Pip (1988).

reproduced in Table 3.1. Post depositional diagenetic conditions applicable to freshwater mollusc populations are outlined in Webb et al. (2007).

There is some variation noted in published studies that evaluate the taphonomic decay mechanisms affecting freshwater assemblages as to which processes are dominant. Potential proposed mechanisms for destruction of unionid shell material include the effects of acidic water proposed by Coker et al., (1921) and prominent in a modern assemblage examined by Cummins, (1994), however, the majority of studies to date attribute shell degradation to physical processes (e.g. Hinch and Green, 1988; Roper and Hickey, 1994; Newell et al., 2007). Strayer and Malcolm (2007) propose a combination of physical processes and contributions from water chemistry more broadly (Ca, pH, dissolved organic carbon and current). In addition to chemical or physical attacks on the shells aragonite, it is also possible for microbial decomposition to damage the shells organic matter (Glover and kidwell, 1993).

It is clear that in the unionid assemblage we examined that a number of distinct processes are acting in concert, at different scales and upon different parts of the shell structure to produce the features illustrated herein. Across the shell exterior, predominantly physical effects are presumed for the initial puncturing and tearing of the periostracum and for initial surface sculpting of the exposed nacreous aragonite layers. A combination of physical abrasion, chemical etching and biogenic microboring effects are then inferred giving rise to distinctive surface pits. Penetration of the interior conchiolin layers is assumed to be hastened where biogenic decay has occurred. On exposed shell interiors, a similar range of processes are assumed to occur, perhaps with chemical processes being of greater prominence. In a study conducted in a nearshore marine setting, Aller, (1982) reports a combination of abrasive loss of periostracum acting in concert with acidic dissolution of underlying aragonitic shell material, with evidence for dissolution around the umbos of living bivalves, including dissolution pits. In addition, chalky areas and boring related pitting are noted. It seems unlikely that any one set of processes would act in isolation as suggested by some previous studies of unionid material and it seems likely that a detailed examination of more unionid samples would yield more evidence for nacre dissolution and shell hosted microboring and epibiota as recorded here.

Overall it would appear reasonable to assume that the preservation potential for the assemblage we describe would tend to be low, with quite pronounced degradation being noted on the shell assemblage even prior to it's deposition. Once emplaced, it is probable that carbonate dissolution in the vadose or phreatic zone would generally prevail and the remaining shell material would be lost (e.g., Bridge et al., 1986). Even if selected samples were preserved it would be unlikely to accurately reflect the original composition of the biocoenosis. Analysis of taphonomic fidelity of lacustrine freshwater molluscan assemblages led Pip (1988) to conclude that "species composition of the sediment shells failed to reflect the composition of the living communities". Furthermore "different sites showed different attrition rates for the same species" as a function of turbulence. However, even despite these limitations, actualistic taphonomic studies may still yield valuable insights even where preservation is unlikely (Kowalewski 1999) and furthermore biostratinomic studies such as ours are particularly appropriate for this line of research (Kowalewski and LaBarbera, 2004). We therefore hope that our study will provide a useful supplement to the sparse existing literature on unionid taphonomy and will promote further research efforts in this direction.

SUMMARY

1) Unionids are subject to a variety of biologically mediated, as well as abiogenic chemical and physical taphonomic processes, both during their life and following death. As a consequence of variation in both environmental and biotic controls, there is significant local variability in the prominence of each of these factors. As a result of these variations, a review of the sparse literature relating to unionid taphonomy reveals a diverse range of decay patterns and preservational styles.

Observations were undertaken on a modern unionid bivalve assemblage from the Saint John River, near to Fredericton, New Brunswick, Canada, in order to document actualistic, early stage taphonomic processes affecting individual bivalve shells in a cool climate, lotic setting.

2) One obvious feature commonly encountered in unionids is damage to the umbonal region of the exterior shell surface. We noted loss of periostracum from this area in all the live and dead unionids that we examined. Removal of periostracum is thought to occur via predominantly physical mechanisms, and to be initiated by processes of puncturing and tearing that are also occasionally observed to occur elsewhere across the shell exterior.

3) Once the periostracum is penetrated, damage to the underlying predominantly aragonitic shell layers then proceeds by a combination of physical, chemical and biological processes. Physical damage is thought to take place mainly via abrasion, and is inferred from the presence of silt and very fine sand sediment trapped beneath periostracum and in pitting features found across the exposed nacreous shell layer.

4) The pit features can preserve circular or ovoid morphologies that closely resemble the boring trace fossil *Oichnus*.

5) Chemical dissolution is also evident, with nacreous aragonite tablets within some pit features displaying moderate to heavy etching.

6) Evidence for biotic degradation of the shells takes the form of microborings, as well as locally attached biofilm and associated microorganisms that are observed

colonising some regions of the organic conchiolin layers within the exposed nacreous shell layers.

7) Damage to the shell interior is also a prominent feature of dead shells, which have lost any residue of their soft tissue. Laterally extensive bulk removal of shell material by chemical dissolution appears to be the most significant mechanism of interior shell aragonite loss noted in our assemblage. Some pitting and hole features, with *Oichnus* like morphologies comparable to those previously described on the shell exteriors, are also evident on the interior surfaces of the shells.

8) Although a variety of macroscopic epibionts have been noted on unionids, they are not thought to be significant agents of taphonomic decay. No evidence for radulation traces was noted in our samples and it appears unlikely that either harvesting of shell aragonite, or grazing of microorganisms by gastropods could have been agents of shell decay in our assemblage. Similarly there was no evidence for successful macrofaunal predation in our assemblage.

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CHAPTER 4

MICROBORING IN A FRESHWATER FLUVIAL UNIONID BIVALVE SUBSTRATE[1]

INTRODUCTION

Organisms that create euendolithic microborings are a noted component of present day marine ecosystems, where they exploit both live and dead biogenically derived carbonate substrates, in addition to their occurrence in lithic substrates of purely abiogenic origin (e.g. Mao Che et al., 1996; Schönberg and Tapanila, 2006; Wisshak et al. 2011; Wisshak, 2012 and references therein). Potential selection pressures favouring the adoption of a boring habit are outlined and discussed in Cockell and Herrera (2008). Since, by definition, borings can only occur in hard substrates they tend to be taphonomically resilient structures, and, as such, microborings are often found preserved in the marine rock record (e.g. Golubic et al., 1975; 1981; Radtke, et al. 1997; Schönberg and Tapanila, 2006; Wisshak and Tapanila, 2008 and references therein). To date however, there is a relative paucity of published research addressing microborings within either lentic (still water, e.g. pond, lake or swamp), or lotic (moving water, e.g. stream and river), freshwater environments or their associated preserved sedimentary deposits (Schneider et al., 1983; Anagnostidis and Pantazidou, 1988; Hagan et al., 1998; Schneider and Le Campion-Alsumard, 1999; Schönberg and Tapanila, 2006; Tribollet et al., 2008). Several authors have approached the phenomenon of microboring taking place within stromatolites and other microbial laminate substrates (e.g.

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[1] *A version of this paper has been accepted for publication. Lawfield, Gingras and Pemberton. Ichnos.*

Macintyre et al., 2000; Reid et al., 2000), together with their associated biofilms (predominantly surficial structures of irregular sheet like morphology that are formed from a combination of prokaryotic and/or eukaryotic cells embedded in a matrix of polysaccharide dominated extracellular polymeric substances (EPS) (e.g. Wingender et al., 1999; Wolfaardt et al., 1999; and Flemming et al., 2000)). Biofilms are widely reported in terrestrial settings, with the weathering of lithic substrates relating to conservation of buildings and monuments comprising the bulk of such research (e.g. Crispim and Gaylarde, 2005). To the best of our knowledge, the possibility of an association between biofilm and boring activity taking place in freshwater river or lake environments has not been previously investigated.

Unionid bivalves are an established (Chamberlain, 2004) and prominent component of freshwater ecosystems (e.g. Strayer, 2008; Vaughn et al., 2008; Cummings and Graf, 2009 and references therein) and have recently been found to act as a host for euendolithic microorganisms (e.g. Hagan et al., 1998 and references therein) and in particular cyanobacteria (e.g. Tribollet et al., 2008). We undertook a detailed examination of recent unionid bivalve samples collected from within the channel of the freshwater Saint John River, from Fredericton, New Brunswick, Canada (45^0 58′ N, 066^0 42′ W) (Figure 4.1), in order to document some of the diversity of microboring structures encountered in this setting. A number of unionid bivalve samples were collected from an assemblage of dead shells that had been revealed and made accessible by a significantly reduced water level in a dam controlled reach of the Saint John River. These samples, identified as members of the unionid bivalve species *Elliptio complanata* (Lightfoot, 1786) (Clarke, 1981), form the basis for the present study (Figure 4.2).

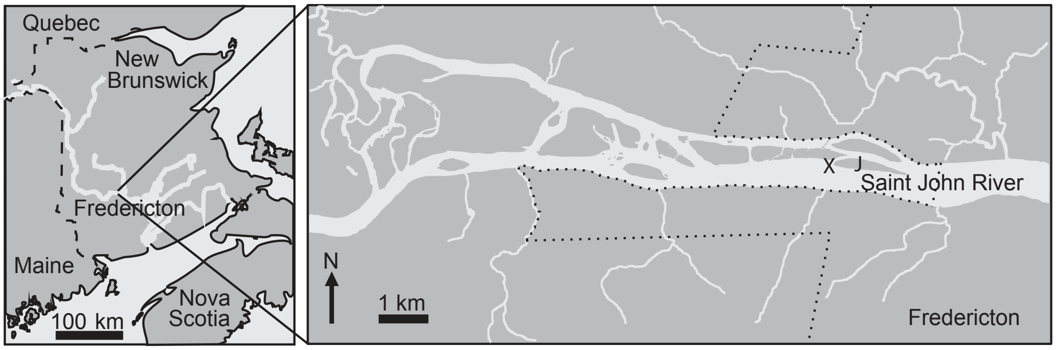

Figure 4.1. Locality map, depicting the course of the Saint John River through New Brunswick, Atlantic Canada. The inset shows the sample collection site (X), adjacent to the western end of Jewett Island (J). The dotted line demarcates Fredericton city limits (modified after Lawfield and Pickerill, 2006).

The location of the study site gives rise to a humid continental climate, with a daily mean air temperature variation between -9.3 °C in January and 19.2 °C for July (Wikipedia, 2006). This results in a seasonal ice cover lasting several months. Water-chemistry analysis conducted during the ice-free period reveals an average pH of 7.7 and alkalinities of 25-102 mg/L $CaCO_3$ (R.A. Curry, cited in Cunjak and Newbury, 2005). Despite draining into a basin with a pronounced tidal range, the study site is at a sufficient distance upstream from the coast to avoid any possibility of saline influence, and in addition, the possibility of a saltwater incursion is further mitigated by the presence of two bedrock sills situated towards the river mouth. At the location where the sample collection was undertaken, the Saint John River is displays a locally anastomosing aspect in plan view, with a mostly continuous main river channel of approximately 500 metres width. A series of islands and narrower channels located to the northern side of the main channel comprise an additional 200 metres of channel confined flow (Figure 4.1). Throughout the duration of the study, the water level, which is locally dam controlled by the Mactaquac Dam 13 Km upstream from the study site, had dropped by a metre compared to its usual level. This enabled safe and

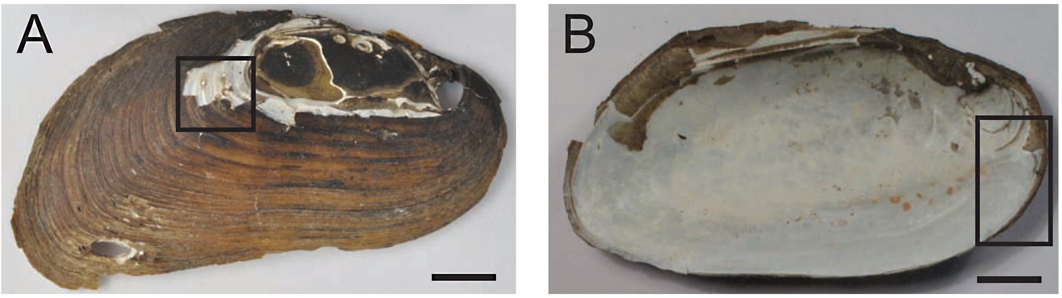

Figure 4.2. Photographs depicting samples of *Elliptio complanata*, the unionid bivalve investigated in this study. **A)** Exterior view of valve, illustrating characteristic taphonomic decay, including physicochemically generated pitting structures, as described in chapter three. Inset indicates location of scanning electron microscopy sample illustrated in greater detail in figure 4.8. **B)** Interior view of valve. Inset indicates location of scanning electron microscopy sample illustrated in figure 4.5. Scale bars = 1 cm.

straightforward access to the sampling locality situated on the bank of Jewett Island immediately adjacent to the main river channel, in the shallow waters of the main channel itself out to a working depth of 0.5 m (that would represent a normal water level of 1.5 m) and on a sandbar that joins Jewett Island to one of the neighbouring islands, the sandbar having been subaerially exposed as a consequence of the fall in water level. The river locally has a velocity of approximately 1.5 m/s at the centre of the main channel. The rivers mean annual discharge of 1110 m^3/s is not evenly distributed but peaks during the April and May snow melt, despite precipitation being evenly distributed throughout the year (Cunjak and Newbury, 2005). The sandbar, riverbank and riverbed are composed of unconsolidated quartz dominated siliciclastic sediments, principally of sand grade for the subaerially exposed and shallow water depth portions, but rapidly and progressively increasing to pebbles and cobbles for the riverbed of the main river channel.

The shell samples that we collected were either newly subaerially exposed by the emersion event (for a period of between hours and days) or were obtained from shallow water out to a depth of 0.5 m, beyond which point there was an absence of visible unionids, the current velocity increased and a shift towards a substrate possessing markedly larger grain size took place. From their condition, the collected shell samples, which occurred in an articulated state and appeared to have been subjected to relatively limited transportation, are assumed to be a few years postmortem. Further details of the collection locality, including additional observations on its climatic and sedimentologic situation are described in Lawfield and Pickerill (2006).

METHODS

Field collection was undertaken, with samples collected by hand from a unionid thanatocoenosis that had been exposed subaerially on a sand bank and in the shallow (less than 0.5 m depth) water of the adjacent river channel. Live specimens were avoided, sampling being instead restricted to empty shells. At this location these were all found to be articulated and had been subjected to varying degrees of dissolution and abrasion (described in detail in chapter three).

Following collection, the bivalve shells were air dried at room temperature. Several samples, exhibiting a range of taphonomic deterioration were selected for scanning electron microscopy (SEM) imaging. These were first photographed with a digital camera. Preparation for SEM imaging was then undertaken. In order to enable the specimens to fit the SEM stubs, regions of interest were identified and the shells were then cut to size by hand with a hacksaw (Figure 4.2, see insets and also figure 4.4A). In order to fully observe both the distribution and variety of any microbioerosion that might be encountered, samples were selected for viewing on both their inner and outer valve surfaces as well as in radial section, through the successive shell layers (Figure 4.3 and see also accompanying text in the description section). The samples illustrated in figure 4.2 were to be imaged in plan view across portions of both the interior and exterior shell surfaces (with detailed images seen in figures 4.5 and 4.8 respectively). In addition, in order to obtain images displaying a full radial section from the umbo to the shell margin, and any structures that this might reveal, additional preparation was undertaken on the sample in figure 4.4A, with an initial cut first being created along the line of growth. To allow imaging through the full thickness of the shell, and prevent any features from being obscured by either overlying shell layers or by damage from the sawing process, an inclined broken edge was then superimposed along the line of the saw cut using pliers. The mounted samples were sputter coated with gold and then imaged at 5.0 kV with a Jeol 6301F Field Emission scanning electron microscope.

Following successful imaging of microboring features visible on the exposed exterior shell surfaces, the samples already known to host microborings were subjected to epoxy resin casting, using a modified version of techniques described in Nielsen and Maboe (2000), who themselves provide a simplified methodology, building on and incorporating elements first pioneered for microboring material by Golubic et al., 1970. The following methodology was employed to generate casts. First, to prevent the epoxy resin from completely enclosing the samples, the rear of the samples (the opposite face to that previously examined and known to host microborings) was immersed in heated thermoplastic glue (Crystalbond^{TM} 509, heated to 90 °C on a hot plate). The thermoplastic glue is chemically inert with respect to carbonate substrates and of sufficiently high viscosity to avoid any risk of penetration and clogging of the boring network or other shell structural

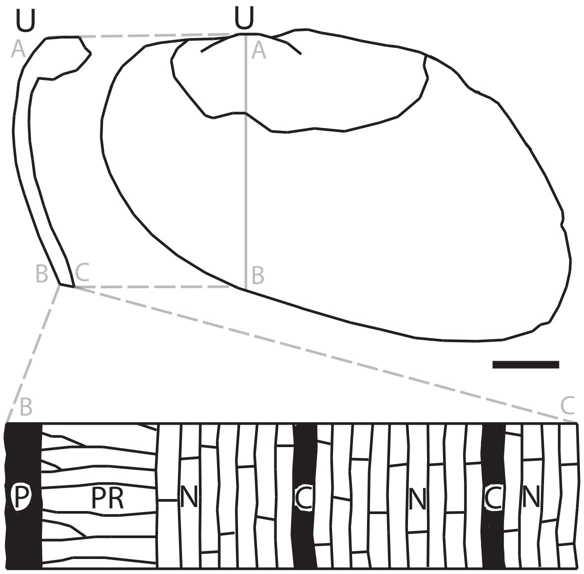

Figure 4.3. Diagrammatic representation of unionid shell structure, indicating the relative positions and orientations of each shell layer. **Upper images)** Depict the shell shape in lateral view **(top right)** and apical plane cross-sectional view **(top left)**, the pale grey line A-B denoting the line of section. U = umbo. The line surrounding the umbonal region, depicted in the top right figure approximately demarcates the umbonal area affected by loss of the exterior periostracum layer, with predominantly intact periostracum occurring across the rest of the shell surface. Scale bar for both images = 1cm. In the lower cross sectional view **(bottom)** where B-C denotes the line of section, black regions represent areas of organic proteinaceous conchiolin and white areas represent crystalline aragonite. From the shell exterior, progressing inwards; P = periostracum, composed of conchiolin; PR = prismatic aragonite layer; N = nacreous aragonitic layers; C = conchiolin. The lower cross sectional view is a representative image and doesn't illustrate the prolific number of nacre layers, which is typically an order of magnitude higher than depicted here. Lower cross sectional view not to scale.

features. At the selected temperature, the glue is viscous enough to support the sample and prevent its complete immersion. The orientation of the samples relative to the thermoplastic glue was carefully selected to allow the resin to subsequently access, cover and penetrate the full extent of the surface known to host the microborings. The thermoplastic glue was allowed to cool and set. Resin impregnation was then undertaken using Epo-Tek® 301 mixed at room

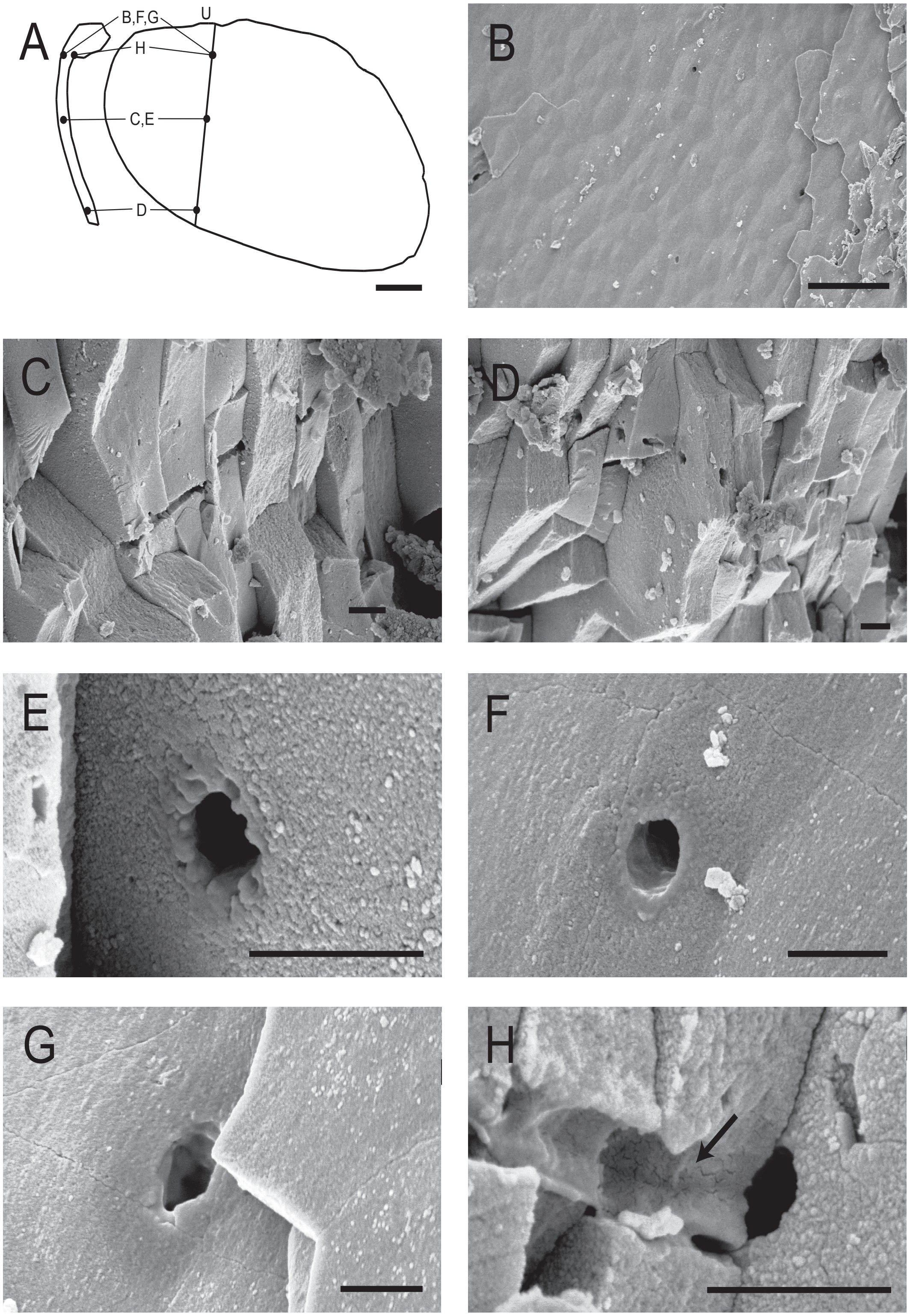

Figure. 4.4. Images of shell microborings in *Elliptio complanata* (Unionoida). **A)** Diagrammatic view of exterior surface of left valve, showing location of umbo (U) and radial section illustrating dorsal-ventral alignment of prepared and examined

section, in addition to approximate locations for images B-H (arrowed). **B-H)** Scanning electron microscopy images of the nacreous shell layer. **B)** Exterior planar surface view of exposed nacreous layer in umbonal region, illustrating density and distribution of microborings. **C)** Characteristic c-axis alignment of microborings, penetrating through and normal to multiple tablets in a fractured radial section of the bivalve. **D)** Fractured radial section of *Elliptio complanata* demonstrating an inclined microboring alignment. **E)** Planar view showing relationship between microboring and surface of nacre tablet. **F, G)** Detail views from within image B, showing variation in cross-sectional morphology of the microborings and their lining ornamentation. **H)** Detail of ornamentation of interior within microboring (note sinuous furrowing indicated by arrow). Scale bars: A = 1 cm; B = 10 μm; C-H = 1 μm.

temperature, according to the standard recommended proprietary instructions. The mixed resin was applied to the sample, with penetration being aided by the use of a vacuum chamber. Care was taken to enable sufficiently rapid penetration of the sample by the resin to avoid boiling the resin. The vacuum was applied and released several times until no visible bubbles were present in the resin (with ten repeated applications of the vacuum being undertaken to reach this stage). The resin was then left to cure for 24 hours. The thermoplastic glue was then removed from the bivalve shell sample and the attached resin cast by briefly heating on a hot plate to 85 °C. The final residue of thermoplastic glue was removed by quick submersion in acetone that was flushed from the surface of the sample with a low-pressure air hose. This left the shell sample with attached and impregnated epoxy resin. In order to reveal the cast for imaging purposes, the calcareous shell material was removed by acid dissolution of the aragonite via immersion in 10 % by volume CH_3COOH (acetic acid). Digestion of the organic periostracum and interior shell layer conchiolin material was accomplished by immersion in 7.5% NaClO (Sodium Hypochlorite bleach). The sample was carefully immersed in distilled water between each stage of acid and bleach immersion. Two stages of dissolution and digestion were conducted, totaling 96 hours in the acetic acid and

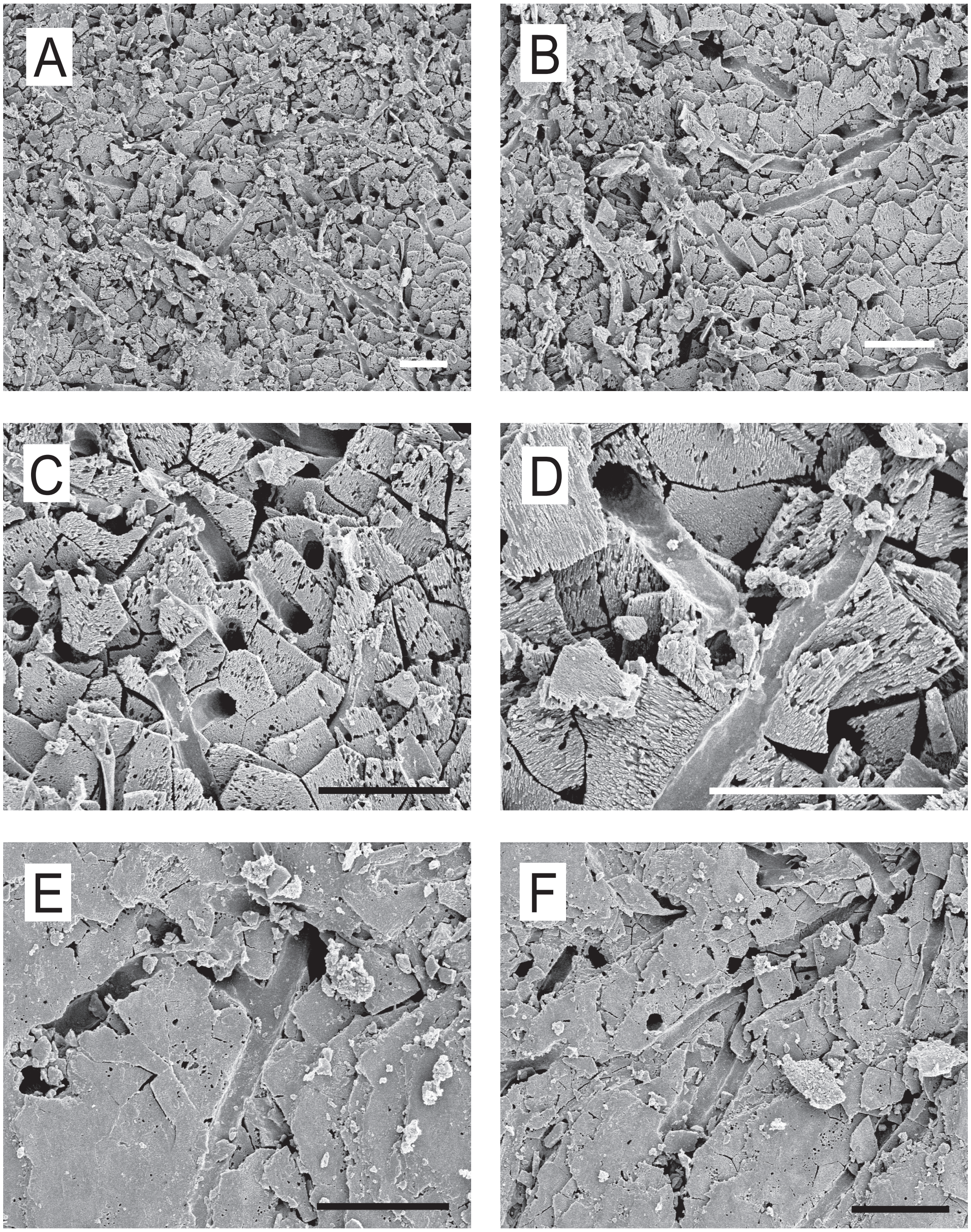

Figure 4.5. Scanning electron microscopy images of the nacreous interior shell layer of *Elliptio complanata* (Unionidae), illustrating microboring. Moderate chemical etching of nacreous aragonite tablets is also apparent. **A-B)** Overview images showing density and random orientation of microboring features. Note local sheets of extracellular polymeric substance and coalescing fibrous organic material, orientated parallel or at low angle to the shell interior and often found in association with borings. **C-F)** Detailed views of borings penetrating nacreous aragonite tablets. The lining, surface texture, prevailing orientation parallel or at a low angle to the shell interior and the presence of branching

(E) and coalescing **(F)** are readily apparent. Scale bars = 10 μm.

48 hours in the bleach, resulting in near complete (>95%) loss of shell material. The concentrations proved effective at gradually dissolving and digesting the shell, at a sufficiently slow rate to avoid damage by a too vigorous chemical reaction. Some softening of the resin was observed during the acid immersion, so we didn't carry out a third repeat stage of dissolution and digestion. Once the shell material was removed, the epoxy resin cast was oven dried at low temperature (40 °C). Gold coating and SEM imaging were then undertaken following the same procedure as those employed for the initial examination and direct imaging of the shell samples.

Although systematic examination was undertaken on several additional bivalve samples beyond those figured here, on both their exterior and interior shell surfaces, as well as on cross sections displayed through broken shell edges, only the most prominent and distinct regions of bioerosion, found in the three figured samples and their epoxy resin casts were imaged and these form the basis of the following descriptions.

DESCRIPTION

Overview Of Unionid Shell Structure, Taphonomic Signatures And Boring Structures

In order to better understand both the spatial location and host substrate properties for each of the borings described herein, it is first necessary to have some understanding of the main components of the unionids layered shell structure, including the relative positions of each shell layer (Both the form and relative positions of each of these layers are depicted diagrammatically in figure 4.3). To describe this shell structure verbally, from the shell exterior moving progressively inwards, one first encounters a periostracum layer composed of predominantly proteinaceous organic conchiolin. This is typically of the order of tens of microns thickness (personal observation and Checa, 2000). Immediately below this is a layer of prismatic aragonite crystals, approximately one hundred microns thick that account for approximately 10% of the shell thickness (personal observation and Checa and Rodríguez-Navarro, 2001). The majority of the shells thickness occurs below both the periostracum and prismatic layers and takes the form of a thick predominantly crystalline aragonitic layer, comprising multiple layers of nacre tablets, interspersed with minor amounts of organic proteinaceous conchiolin that occurs in the form of discontinuous sheets. The nacreous shell layer constitutes the majority of the shell thickness (89% in *Unio elongatulus* e.g. Checa, 2000), and is typically of between one and several millimetres thickness, this magnitude varying with both the position across the shell and the preservation state of the shell. Further details of the shell structure of unionid bivalves, including descriptions of the various shell layers and their formation are presented in Taylor et al, (1969); Petit et al, (1980a and b); Petit, (1981); Checa, (2000); Checa and Rodríguez-Navarro, (2001) and Marie et al., (2007).

A variety of features produced by taphonomic deterioration are present throughout the shell and further particulars of these are presented in chapter three. In brief these include puncturing and tearing of the shell periostracum by physical means. Damage to and loss of the underlying aragonitic shell layers also occurs, by physical abrasion as well as via dissolution through chemical etching from the water column. The organic conchiolin layers occurring within the nacreous aragonitic portion of the shell interior are exploited by a range of epibiotic microrganisms, together with associated EPS and biofilms (see later description and discussion). The interior shell conchiolin is also subjected to damage by physical processes in the same fashion as those that affect the periostracum. The outcomes of these processes include exposed shell aragonite, particularly in the umbonal region, as well as a sculpted topography across the exposed aragonite, sometimes resulting in rounded pits with an *Oichnus* like morphology.

It should be emphasised that intensive visual and SEM observations conducted upon multiple bivalve specimens failed to detect any visible boring within either the periostracum or prismatic shell layers of the examined unionid shell samples. Any boring structures described herein are therefore confined to the underlying nacreous shell material, together with its associated organic conchiolin layers.

Borings are noted on both the nacreous shell layer exposed on the interior surface of the dead empty shells (e.g. See figure 4.2B for location of detailed SEM images illustrated in figure 4.5, as well as the accompanying description of the interior surface boring network) and on the upper surface of the nacreous layer, where this has been revealed by removal of the overlying prismatic shell layer and protective periostracum (e.g. See figure 4.2A, for the location of SEM images displayed in figure 4.8; as well as figure 4.4B. Also refer respectively to the descriptions of the horizontal boring network on the upper surface of the nacreous shell layer and the penetrative, simple, steeply aligned tubular borings). Borings are also observed throughout the full thickness of the nacreous shell layer (e.g. Figures 4.4A and C-H, which accompany the description of penetrative simple steeply aligned tubular borings). The exposure of the upper surface of the nacreous layer occurred as a consequence of the destructive properties of both the abrasive physical action of river water and associated sediments as well as chemical etching by dissolution as a result of interaction with the river water. Chemical etching is evidenced by the presence of lattice structures formed on the surfaces and margins of individual nacre tablets. In addition to these documented physicochemical processes there may also have been some additional damage caused by freshwater microorganisms. No evidence of precipitation was noted, either associated with boring activity or independent of it.

The borings noted here include predominantly surficial structures (Figures 4.5 and 4.8), and in addition, some penetrative structures are observed spanning the thickness of the nacreous layer (Figure 4.4). The latter bear an often marked visual similarity to structures described and identified elsewhere as microtubules (e.g. Oberling, 1964 (varied bivalve fauna, marine); Taylor, Kennedy and Hall, 1969 (varied bivalve fauna, marine); Morton, 1978 (*Philobrya munita* from intertidal shores); Siddall, 1980 (*Mytilopsis leucophaet*, estuarine); Waller 1980 (Arcoida, marine); Prezant, 1990 (*Lissarca notorcadensis*, marine); Araujo et al., 1993 and 1994 (*Corbicula fluminea* from rivers); Reindl and Haszprunar, 1996 (varied bivalve fauna, marine); Schwartz and Dimock 2001 (Unionidae *Utterbackia imbecillis* and *Pyganodon cataracta*, pond) and Malchus, 2010 (*Condylocardia*, marine)). However, despite this morphological similarity, evidence of microtubules has not previously been encountered in adult unionids and in addition to this absence of any prior observation (despite many detailed studies of shell structure in unionids (see references above)) the structures we encounter here can also be differentiated on the basis of the presence of a distinctive lining similar to that noted on the dominantly surficial boring structures also described here, as well as due to their displaying a wide variation in diameter, neither of which are characteristics associated with microtubules.

Penetrative Simple Steeply Aligned Tubular Borings

A number of borings are noted spanning the thickness of the nacreous shell layer (Figure 4.4). They occur at a much lower density (Figure 4.4B) than the predominantly surficial structures that are also described here. These tubular borings display a characteristic straight hollow cylindrical morphology, with a prominent lining. Individually the borings display uniform inner and outer thicknesses along their length (measurements being taken at both the inner and outer margin of the lining). Thicknesses for the inner diameter measured across the inner margin of the lining range between 400 and 800 nm for different borings. No examples of branching are observed and similarly cross-cutting appears to be absent, although this would be expected given the low density of the borings. The borings are consistently oriented at a high angle to the plane of the shell crystal structure, either perpendicular to the individual nacre tablets (parallel with the crystallographic c-axis) (Figures 4.4B,C,E-H), or rarely with a slight inclination (Figure 4.4D). Transverse sectional views across the borings generally reveal sub-circular (Figures 4.4E and F) to more irregular (Figure 4.4G) cross sectional morphologies. When the inner surface of the borings is exposed in longitudinal section it generally displays a smooth but lumpy texture, marked by nodular irregularities. Locally a furrowed sinuous ornamentation is also noted on the inner surface of the lining (Figure 4.4H). Borings are generally present at central positions within the individual nacreous crystal tablets. The borings appear to span the entire thickness of the nacreous shell layer, with clear continuity being observed across successive nacreous layers (Figures 4.4C and D).

Surface Boring Network On Shell Interior

The most complex array of borings is observed on the interior surface of the shell (Figure 4.5). This comprises a dense and laterally extensive network of irregularly aligned interconnected tubular borings. The borings are of micron scale, and individually display little variation in thickness along their length (their total length being indeterminate). Within the visible area, borings are noted with cross-sectional thicknesses varying between 1.9 and 2.2 microns. The borings display a distinct lining of approximately 250 nm thickness. The lining is assumed to be organic and appears smooth on both its interior and exterior surfaces.
The majority of the borings appear to run either parallel with, or at a shallow angle to the shells inner surface. A number of more steeply aligned examples are also noted, revealing a distinct circular cross section (e.g. Figure 4.5C). The crystallographic alignment of the nacreous shell structure appears to exert no influence on the orientation of these borings, which are seen to cut across multiple nacre tablets and are present at both the centre and edges of the individual nacre tablets (Figure 4.5). The borings display rare branching, with side branches aligned at a high angle to the main boring (Figure 4.5E). Multiple borings are occasionally observed to coalesce (Figure 4.5F).

Complex, Laterally Extensive Three-Dimensional Boring Network

Epoxy casting reveals a complex branching boring network (Figure 4.6) that is visible at several locations distributed sporadically across the inner surface of both shell samples that were subjected to resin casting. The preserved and visible regions of network are of mm lateral extent and up to several hundred micrometres depth. Each of the main branches of the boring network are generally of between two and five microns in diameter, although on occasion they fall

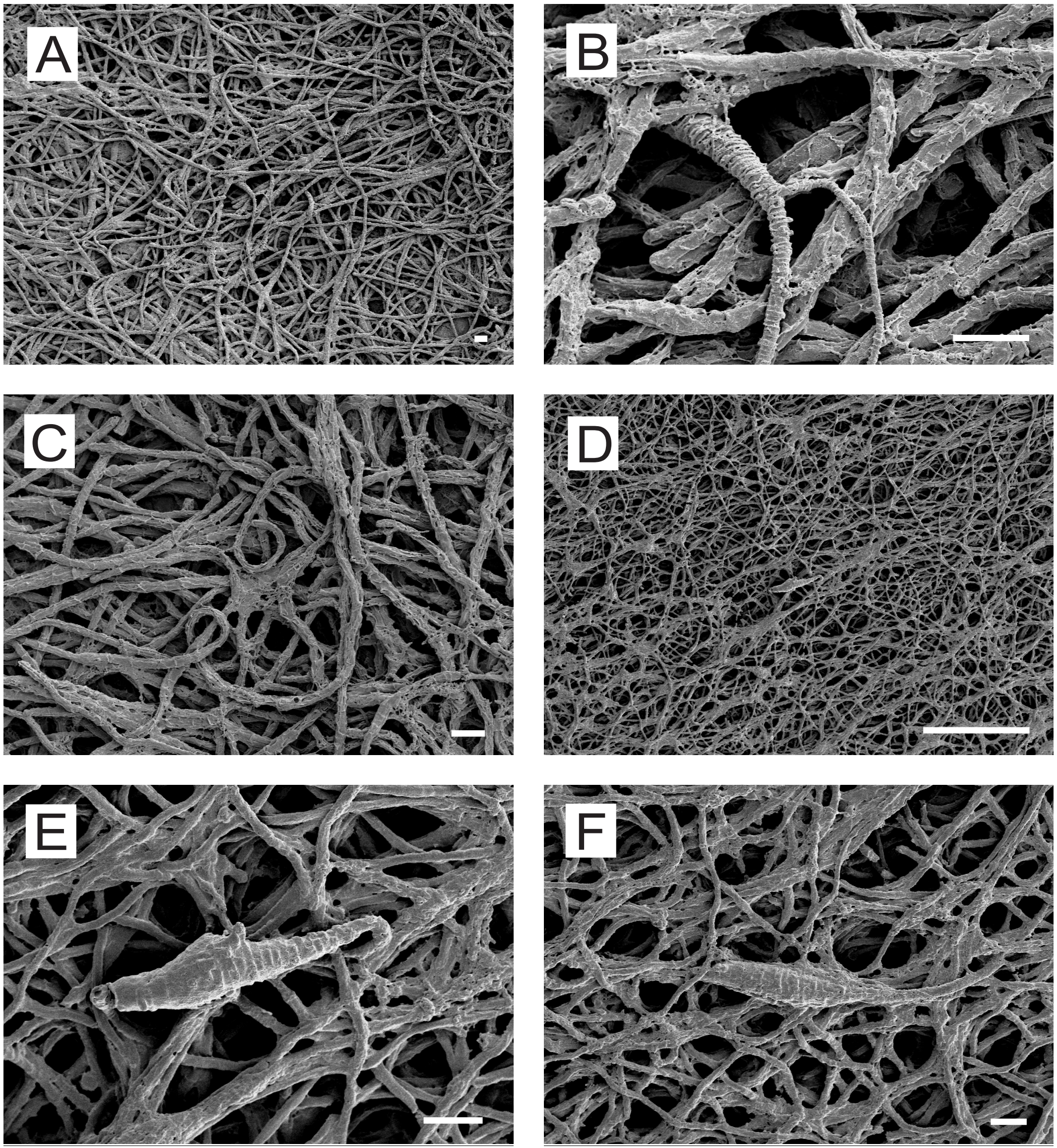

Figure 4.6. Epoxy resin casts of the complex irregular branching microboring network that is distributed sporadically throughout the shell interior **(A-F)**. The preserved and visible regions of the boring network are of mm lateral extent and up to several hundred micrometres depth normal to the shell surface. The main branches of the boring network are generally of between two and five microns in diameter, although on occasion they fall beyond both the lower and upper limits of this range. Individual tubes display branching with y form junctions. Branching is generally limited to two branches per junction, with the separation of two strands of equal diameter, although more complex coalescing of multiple strands is also rarely observed. Occasional discontinuities along the path of individual tubes are present **(e.g. B and F)**, but their form and positioning is consistent with being artifacts of the casting process, as opposed to genuine terminations or breaks in the individual tubes. Apparently randomly distributed features including looping **(C)**

and bulbous nodes of up to 10 micron diameter **(D-F)** are apparent and add complexity to the morphology of the structure. Additional surface ornamentation includes a network of irregular ribs of hundreds of nanometres width, distributed across the surfaces of individual tubes **(e.g. B and C)**. The bulbous nodes visible in **D-F** are marked by an ornamentation of concentric rings that display a more regular form. Scale bars A-C, E, F = 10 μm; D = 100 μm.

beyond both the lower and upper limits of this range. Branches take the form of y branches and are generally limited to two the separation of two strands of equal diameter, although more complex coalescing of multiple strands is also observed. Occasional discontinuities along the path of individual fronds are present (e.g. Figures 4.6B,F), but their form and positioning is consistent with their being artifacts of the casting process, as opposed to genuine terminations or breaks in the individual fronds. Apparently randomly distributed features including looping (Figure 4.6C) and bulbous nodes of up to 10 micron diameter (Figure 4.6D-F) are apparent, adding complexity to the morphology of the structure. Additional surface ornamentation appears to include a network of irregular ribs of hundreds of nanometres width, distributed across the surface of the individual tubes. The bulbous nodes visible in figure 4.6D-F are marked by an ornamentation of concentric rings that display a more regular form.

Simple tunnels or tubes are also apparent on the inner shell surface (Figure 4.7). These are sinuous and either solitary or observed to occur in low-density concentrations, with minimal overlapping. Individual strands are discontinuous presumably as a result of inadequacies in the epoxy resin casting process. The structures are generally a couple of microns in diameter, although finer examples of hundreds of nanometre diameter are also apparent. Only those strands aligned parallel with the shell surface are preserved, and although there is some indication that structures with an alignment perpendicular to the shell surface may also

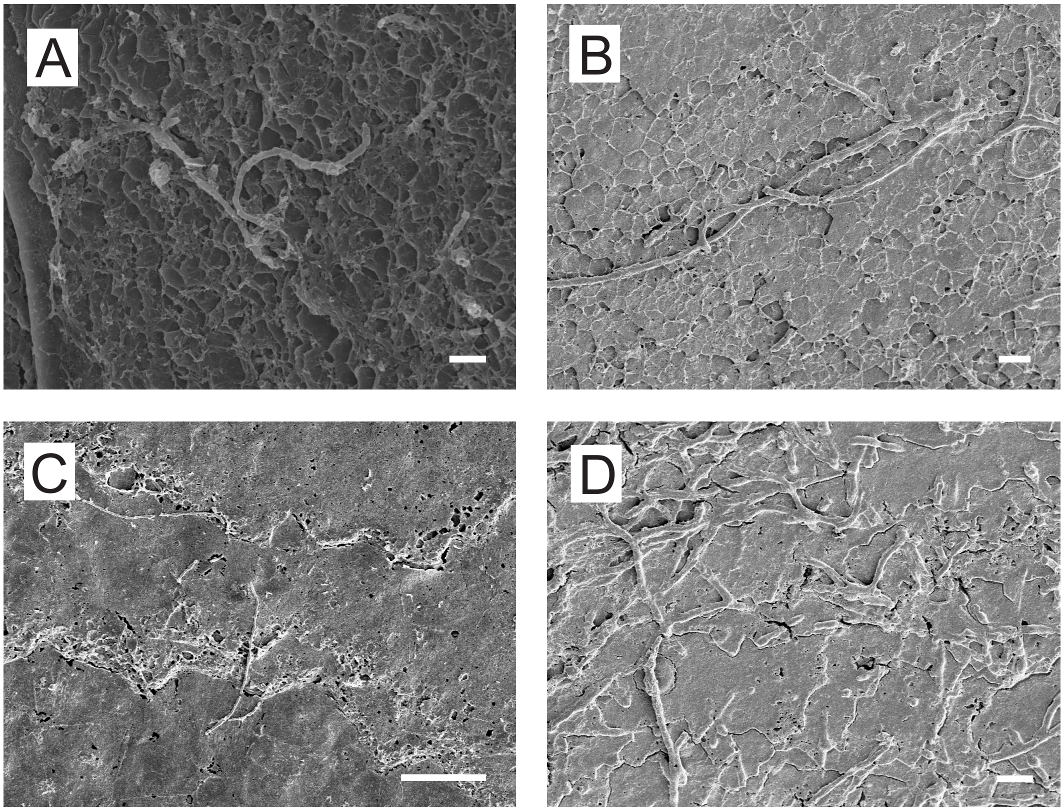

Figure 4.7. Epoxy resin casts of simple tubular surficial microborings are apparent on the postmortem exposed interior surface of the shell. These are sinuous and occur as solitary tubes or low-density concentrations with minimal overlapping. The latter are gradational with the complex irregular branching microboring network (figure 4.6). Individual tubes are discontinuous **(A-D)**, presumably as a result of limitations of the casting process. The structures are generally a couple of microns in diameter **(A-B, D)**, although finer examples of hundreds of nanometre diameter are also apparent **(e.g. D)**. Only those strands aligned parallel with the shell surface are preserved, and although there is some indication that structures with an alignment perpendicular to the shell surface may also occur, their presence is only indicated by blunt terminations, projecting towards the viewer **(e.g. D)**, again due to limitations in casting. Outlines of individual nacre tablets are also evident **(A-D)**. Scale bars A-D = 10 μm.

occur, their presence is only indicated by blunt terminations (e.g. Figure 4.7D), presumably due to issue relating to the casting process. A fine polygonal ridge network is also locally apparent across the shell surface, preserving the outlines of individual nacre tablets.

The structures visible in figure 4.7 appear to be dimensionally and morphologically continuous with those illustrated in figures 4.5 and 4.6. We believe that they represent different densities of boring structures, with further complexity being introduced by variation in both the fidelity of the epoxy resin casting process as well as differences relating to viewing the microboring structures directly and in epoxy resin cast form. The epoxy casting process failed to reproduce the sparse, hundreds of nanometre diameter borings aligned normal to the shell surface that were visible in the samples prior to the casting process.

Horizontal Boring Network On Upper Surface Of Nacreous Shell Layer

A complex branching boring network is encountered on the taphonomically exposed upper surface of the nacreous shell layer (Figure 4.8). Although of limited lateral extent, the boring activity observed here is locally intensive. This boring appears to be closely associated with both an organic conchiolin layer and the presence of extracellular polymeric substances (EPS), to a degree that strongly suggests a causal linkage between the three components. Possible mechanisms for the formation of this structure are illustrated schematically in figure 4.9.
The boring consists of a branching pattern of polygonal depressions or pits of presumed biogenic origin, eroded into the conchiolin layer. The pits penetrate the full thickness of the conchiolin, which is of micron scale and are separated by ridges formed by resistant and preserved sections of conchiolin. Individual pits have between three and six sides, with four or five sides being most common. The ridges separating the pits are themselves between six and seven microns wide and serve to separate pits whose dimensions vary, between ten and twenty five microns along their longest axis. When describing the ridges in cross section,

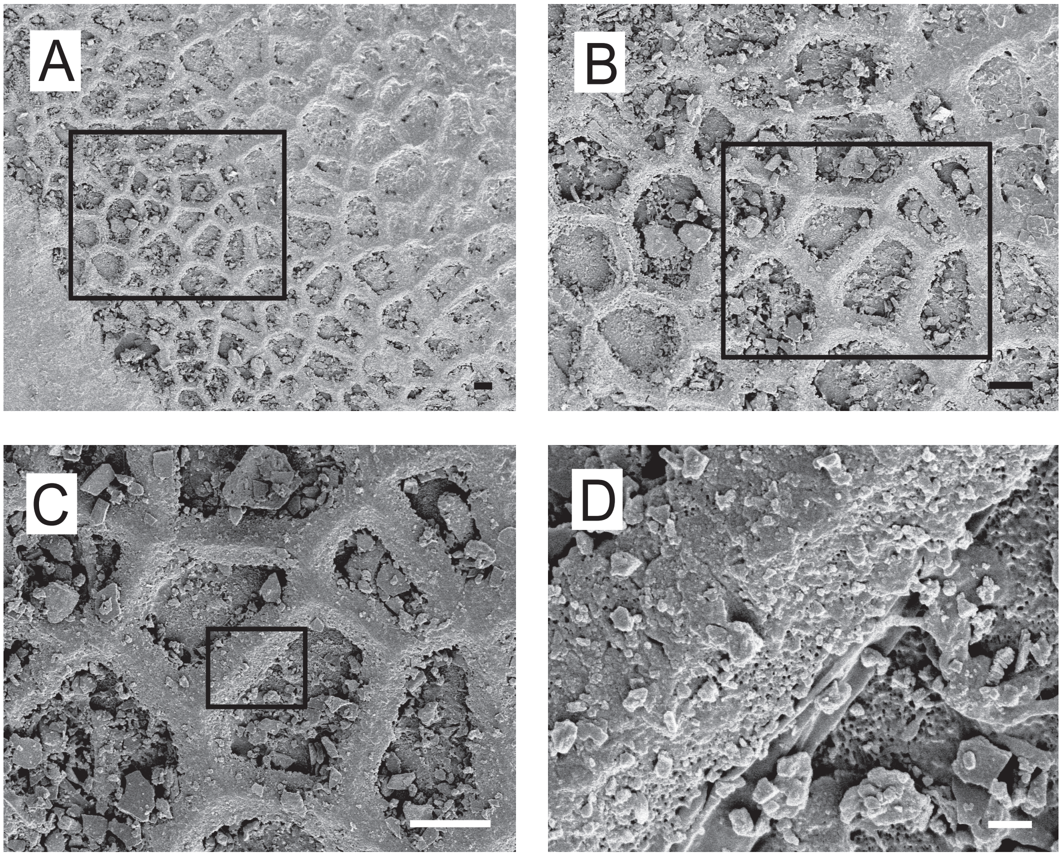

Figure 4.8. Scanning electron microscopy images of microboring affecting an organic conchiolin layer within the nacreous shell layer of *Elliptio complanata* (Unionidae) exposed at the shell surface by taphonomic decay illustrated in figure 4.2. **A)** Progressive degradation of the surface is observed, from unaltered material (left) through an irregular polygonal ridge network (centre) to a continuous, smooth, irregular extracellular polymeric substance (EPS) dominated biofilm surface (right)(Inset box marks location of 5B). **B)** Detail of boundary between moderately (left) and heavily degraded shell material, in which loose nacreous aragonite tablets are enveloped in EPS (right) (Inset box marks location of 5C). **C)** Detail of ridge network. Dislodged nacreous aragonite tablets are clearly visible, together with localised EPS (Inset box marks location of 5D). **D)** Detail of fibrous EPS, similar to examples illustrated in figure 4.6. Scale bars A-C = 10 μm; D = 1 μm.

they taper downwards on their upper surface towards the pits and in addition they are noted to be undercut. Some of the undercutting is associated with the presence of fibrils, which are presumably of fungal or cyanobacterial origin (e.g. Figure

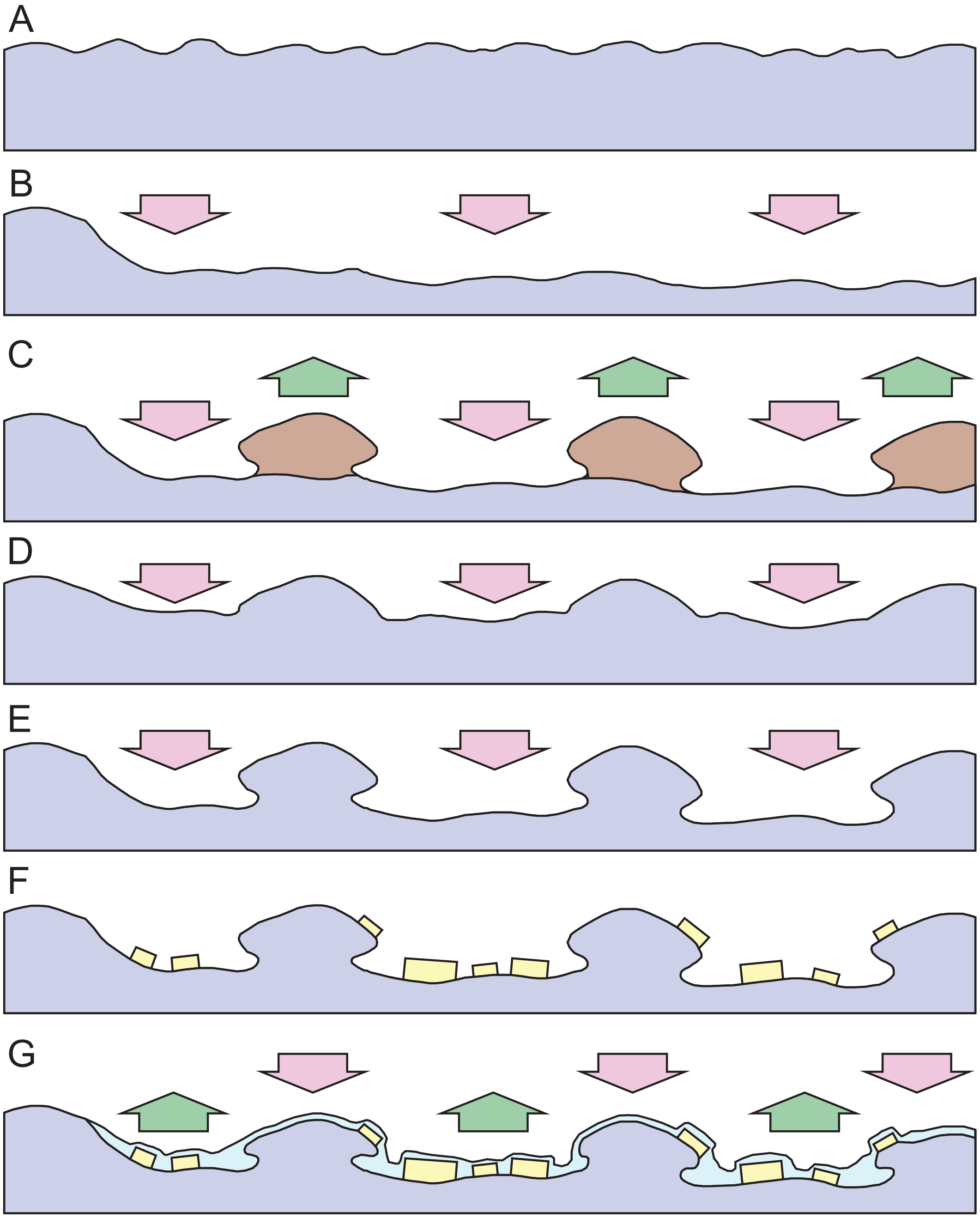

Figure 4.9. Schematic illustration depicting two possible scenarios for the development of the polygonal ridge network in the shell conchiolin layer substrate depicted in figure 4.8. The substrate is an initially intact conchiolin layer **(A)**. One of two possible scenarios may occur, one of which is the progressive degradation of the conchiolin layer **(B)** accompanied by simultaneous or subsequent development of cyanobacterial, cyanophyte or fungal material **(C)**. An alternative scenario is that of the progressive removal of material from the conchiolin layer **(D)**, leaving a resistant network of raised ridges **(E)**. In either event, once formed, the ridge network is subjected to the accumulation of debris,

including nacreous aragonite tablets **(F)** and is itself gradually eroded, simultaneous with the growth of an extracellular polymeric substances (EPS) containing biofilm **(G)**. Purple = Organic material; Brown = newly grown cyanobacterial, cyanophyte or fungal material; Pink arrows = decay and removal of material; Green arrows = growth and addition of material; Yellow = organic and inorganic debris; Pale Blue = EPS and biofilm. Schematic diagram, not to scale.

4.8D). The region of prominent pitting grades laterally in one direction into the exposed but non-bored conchiolin sheet and in the opposite direction is marked by progressive deterioration until the individual ridges lose definition and prominence as the borings grade into a biofilm sheet and in particular extracellular polymeric substance deposits.

Biofilm And Extracellular Polymeric Substances

Exposed shell surfaces, particularly those that include organic conchiolin layers are frequently noted to act as a substrate for an epibiotic biofilm (Figure 4.10). These biofilms are often, although not universally found to occur in association with boring activity (e.g. Figures 4.5 and 4.8), although by no means being confined to areas where boring is present. The biofilms display their greatest thickness, complexity and extent when found inhabiting the organic conchiolin shell layers that are exposed within pit shaped depressions with curving walls that sometimes display an *Oichnus* like morphology. These pit structures occur across the taphonomically decayed exterior shell surface, are thought to be of predominantly physicochemical origin and are the subject of more detailed description and discussion in chapter three. The observed biofilm assemblages display marked variation in composition, being locally dominated by concentrations of pennate diatoms (Figure 4.10A). Other components of the biofilm include cyanobacterial or fungal sheets and filaments (e.g. Figures 4.10B

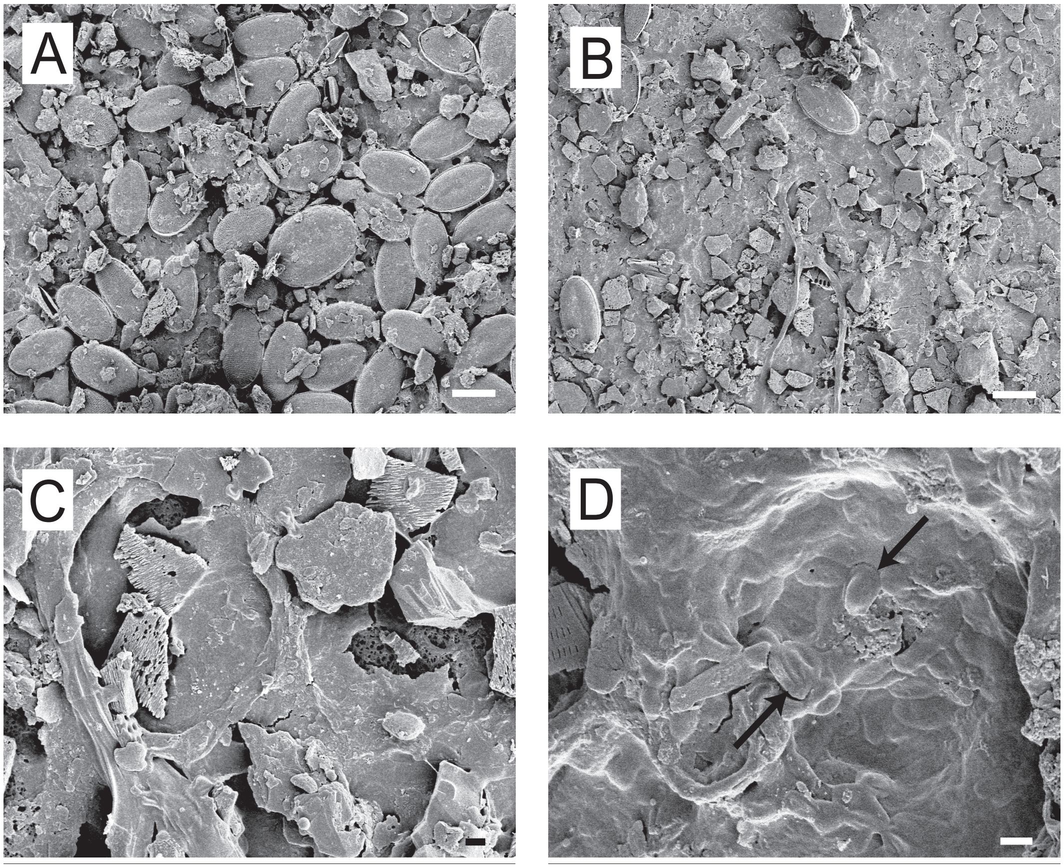

Figure 4.10. SEM imaging reveals the localised presence of an epibiotic biofilm inhabiting the organic conchiolin shell layers exposed within physicochemically generated pit structures that are present across the taphonomically decayed exterior shell surface. The biofilm assemblage displays marked variation in composition, being locally dominated by concentrations of pennate diatoms **(A)**. Other components of the biofilm include cyanobacterial or fungal sheets and filaments **(e.g. B and detail C)**, in addition to amorphous extracellular polymeric substances (EPS) that generally form the majority of the biofilm **(all images)**. In addition to these major components, the forms of bacteria covered by EPS can also be distinguished **(arrowed in D)**. Individual nacreous aragonite shell tablets dislodged from the shell surface as well as lithic fragments are locally incorporated in the biofilm **(most clearly visible in C)**. Scale bars A,B = 10 μm; C,D = 1 μm.

and C), as well as the amorphous extracellular polymeric substances that generally comprise the majority of the biofilm structure (exemplified in Figure 4.10C and D). In addition to these major components, the forms of bacteria covered by EPS can also be distinguished (Figure 4.10D). Individual nacreous aragonite shell tablets dislodged from the shell surface as well as lithic fragments are locally incorporated in the biofilm.

DISCUSSION

Causative Organisms

The presence of microborings in freshwater bivalve shells and the diversity of possible progenitors that could theoretically create such structures was first discussed in Hagan et al. (1998), however it wasn’t until a decade later that Tribollet et al. (2008) documented the presence of *in situ* eundolithic organisms in the form of the cyanobacterium *Plectonema terebrans* occupying a unionid bivalve substrate. Whilst the full diversity of cyanobacterially generated structures isn’t assessed in their paper, which is not primarily a descriptive work, some examples of the range of morphology of *Plectonema* colonies, as revealed by acid dissolution, are figured. Putative microboring activity is also reported as being preserved in unionid fossils of Cretaceous age from the mixed siliciclastic rocks of the Fort Union Formation of North Dakota, U.S.A. (e.g. Burton-Kelly, 2008). These are however of markedly different morphology to the structures we observed and are several orders of magnitude larger, suggesting an unrelated producing organism, or perhaps an entirely different cause of formation. Although in our study we were unable to unambiguously identify the causal trace making organisms associated with the described microborings, the morphological similarity and comparable size distribution of borings illustrated in figures 4.4 and 4.5 to those figured in Tribollet et al. (2008) suggests that they may be attributed to a cyanobacterial progenitor, although it is not possible to exclude fungal or cyanophyte growth as an additional or alternative causal mechanism. Borings produced in marine settings represent a more established field of study and are figured in several published studies, including Glaub and Vogel, 2004, Glaub et al., 2007, Vogel and Brett 2009, Wisshak et al. (2011), and again our assemblage is consistent with their figured cyanobacterial, cyanophyte or fungal microboring morphologies (see also our discussion on biofilm). In addition to the previously discussed microorganisms, the reticulate pattern of the horizontally oriented boring network depicted in figure 4.8 bears some morphological similarity to networks constructed by the Myxomycetes (social amoebae/slime moulds).

Biofilm And Extracellular Polymeric Substances (EPS) And Their Relationship To The Boring Structures

Extracellular polymeric substances are a prominent (50-90% of total organic matter) component of the less precisely defined term ‘biofilm’, within which they are “responsible for the morphology, structure, coherence and physico-chemical properties” (Flemming et al., 2000 and references therein). The structure and function of EPS and biofilms more generally are the subject of several reviews including those by Wingender et al., (1999), Wolfaardt et al., (1999), and Flemming et al., (2000). Biofilms are defined by Costerton, (2007) as “a multicellular community composed of prokaryotic and/or eukaryotic cells embedded in a matrix composed, at least partially, of material synthesized by the sessile cells in the community.” e.g. EPS. Various definitions for extracellular polymeric substance are reviewed in Wingender, et al. (1999) and their broad use of the term as microbially derived biosynthetic polymers including “polysaccharides, proteins, nucleic acids, (phospho)lipids, and other polymeric compounds” in the “intercellular spaces of microbial aggregates” is continued herein. Biofilms and EPS may be created by a variety of microorganisms including bacteria and fungi (Wingender et al., 1999).

Several authors have approached the phenomenon of microboring activity taking place within stromatolites and other microbial laminate substrates, together with their associated biofilms (e.g. Macintyre et al., 2000; Reid et al., 2000). The presence of the boring cyanobacterium *Solentia* sp. has frequently been noted in these marine settings. Currently no published work exists examining the possibility of the potential for similar relationships occurring in freshwater settings. Although biofilm related bioerosion is widely reported in terrestrial contexts, such as the weathering of lithic substrates relating to conservation of buildings and monuments (e.g. Crispim and Gaylarde, 2005), the presence of discrete microscopic or macroscopic boring is generally either overlooked or simply absent. Our results demonstrate a close spatial relationship between biofilm growths and microboring in freshwater carbonate settings, however further study would be required to demonstrate whether a causal relationship exists between these structures.

Biofilms and EPS are produced at significant metabolic expense, however they prove beneficial to the organisms that create them. In the context of the present study, the most pertinent reasons for EPS and biofilm production include adhesion to surfaces, protection, communication and nutritional benefits (including both storage and exploitation of nutrient sources) (Wingender et al., 1999; Wolfaardt et al., 1999, and references therein). The literature on aquatic biofilm formation suggests that diatoms are the most common and abundant early coloniser during the formation of freshwater biofilm (e.g. Jackson and Jones, 1988), whilst raphid diatoms are described by Callow (2000), as "instrumental in the primary colonisation of submerged substrata." These observations are entirely consistent with the compositions observed in our biofilm assemblages, within which diatoms often (although by no means universally) form a prominent component, together with other organisms which are noted to occur as the organic conchiolin layers of the nacreous shell layer are progressively exploited. The limited thickness of the conchiolin layers within the nacreous portion of the unionid shells limits the extent of development of the biofilm, and this is consistent with Costerton (2007) remarking that "Biofilms form when planktonic cells encounter organic nutrients, and they develop to thicknesses that reflect the amounts of nutrient available at that site". Similarly Romaní, (2010), observes that the proportion of fungal biomass is closely tied to substrate, being found to be rare (<1% biomass) in epilithic biofilms and more abundant on organic substrates (>80% biomass), which would lend support to a fungal interpretation for at least some of the fibrous borings, although by no means precluding the possibility that they might be of cyanobacterial origin.

SUMMARY

1) Samples of the unionid bivalve *Elliptio complanata* were collected from the channel of the freshwater Saint John River, from Fredericton, New Brunswick, Canada. Scanning electron microscopy imaging of prepared shell samples revealed an assemblage of microborings. No borings are noted on the periostracum or prismatic shell layers. Boring structures are instead confined to the underlying nacreous aragonitic shell material, together with its associated organic conchiolin layers.

2) We observed three distinctive morphologies of microborings, occurring in both

the organic conchiolin and calcareous nacreous layers of unionid bivalve shells:

A) A reticulate network of pits established on an organic conchiolin sheet within the nacreous shell layer.

B) Simple tubular borings present in the calcareous nacreous aragonitic portion of the shell, with individual isolated tubes of hundreds of nanometres diameter perpendicular to the shell surface. These structures penetrate successive layers of nacre tablets, generally coinciding with the crystallographic c-axis although whether there is a mineralogical control on their distribution is a matter of speculation. It is worth noting that these structures, although clearly visible during direct SEM imaging of the shell surfaces were not present in the epoxy resin casts. Given their fine diameter and evident fragility this may represent a limitation of this established technique and certainly represents an area where care should be taken during sample preparation, since it suggests that some microborings may be overlooked during the study of assemblages where epoxy resin casting is the sole observational technique employed.

C) A complex network of micron diameter tubes, with multiple orientations, in addition to rare coiling and branching. These borings are present in aggregations across relatively large areas of the calcareous nacreous shell layers and are by far the most prolific borings in our assemblage.

3) Cyanobacteria, cyanophytes and fungi are all commonly associated with microboring in marine settings, where they often create tubular structures of similar morphology to those noted in our assemblage. Given our available dataset it is not possible to ascertain which of these taxa might be responsible for the

creation of our microboring assemblage. Similarly, although Myxomycetes are encountered in freshwater settings and can show morphologies similar to the structure we noted on the organic conchiolin layer, further work would be required to determine whether they might have created the structures we observed.

4) We also noted an apparent association between extracellular polymeric substances (EPS) and biofilm with bacterial, diatomaceous and filamentous components and some of the boring styles, although it is inconclusive as to whether a causal relationship can be demonstrated between these components.

5) Our study demonstrates that euendolithic microorganisms are an active component of freshwater ecosystems. The presence of several previously unreported and distinctive boring styles occurring in unionid bivalve shell substrates suggests that freshwater microboring represents a significantly underexplored avenue of ichnological study, with significant potential for future research. Potential avenues of enquiry could include establishing the variety of boring taxa in freshwater settings, the range of biogenic and abiogenic substrates that they exploit and the range of factors that influence their distribution. In addition, it is possible that euendolithic organisms may exert a deleterious effect on the living communities of the host substrate organisms, as well as contributing to the taphonomic decay of their postmortem shell material. Given appropriate preservational conditions the possibility of such features being present in the rock record could also be investigated.

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CHAPTER 5

WILLING HOSTS? EXPLOITATION OF THE UNIONID BIVALVE *ELLIPTIO COMPLANATA* AS A SUBSTRATE BY EPIBIOTIC MICROBIAL MATS IN THE SAINT JOHN RIVER, NEW BRUNSWICK, CANADA[1]

INTRODUCTION

Although themselves confined to freshwater settings, unionid bivalves (freshwater mussels) possess most of the characteristics shared by the majority of marine basibionts. As defined by Wahl (1989), basibionts are organisms that provide a substrate for populations of attached epibiotic organisms. As summarised by Wahl and Olaf (1999), the characteristics of ideal basibiont organisms include comparatively large size, longevity, a slow moving epibenthic habit and “physiologically inactive body surface areas” (in this instance a shell). It is therefore surprising that although epibiotic associations have been reported previously for unionids, the majority of such published works have focused solely on the impact of invasive *Dreissena* (Zebra Mussel) infestations (see for example Nichols, 2002 and Hörmann and Maier, 2006 and references therein). As a consequence there are relatively few publications addressing the wider community of unionid hosted epibiota (e.g. Beckett et al., 1996, Spooner, 2002, Vaughn et al., 2002 and 2008, Tribollet et al., 2008). Whilst undertaking fieldwork for a study investigating the taphonomic processes that affect unionid shells in a

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[1] *A version of this paper has been submitted for publication. Lawfield, Gingras and Pemberton. Ichnos.*

fluvial setting (see chapter three), a number of live unionids were noted to host an epibiotic microbial mat or biomat. Further details of this association are presented herein.

STUDY SITE

Field observations were undertaken at the western end of Jewett Island, its attached sandbar and within the adjacent channel of the Saint John River, near to Fredericton, New Brunswick (45°58′ N, 066°42′ W). The riverbed is usually obscured from view by the depth of water, however a one metre drop in water level caused by a period of end summer drought during September briefly exposed the study site to view. At this point in its course the river is wide and displays an anastomosing form in plan view, with a predominantly sandy bed except in the centre of the largest river channels where coarser siliciclastic substrates of pebble and cobble grade occur. There is relatively little vegetation in the channels, presumably as a consequence of the strong current. The water velocity at the centre of the main river channel, adjacent to the Jewett Island study site, is approximately 1.5 metres per second. As a result of its width and the correspondingly low levels of shading, rocks on the riverbed are exposed to sunlight, allowing the growth of attached periphyton. Further particulars relating to the climatic, hydraulic and sedimentologic character of the site are provided in Lawfield and Pickerill, 2006; Lawfield et al., in press and chapter three.

METHODS

A visual survey was undertaken along a 100 m stretch of sandbar and adjacent riverbank on both the subaerially exposed sediment and below the new water level, on the riverbed, out to a depth of approximately 50 cm (equivalent to a position of 1.5 metres depth under normal flow conditions). Beyond this depth point, a combination of increased water velocity and a reduction in clarity impeded observation. Observations were undertaken on all microbial mat hosting bivalves encountered in this accessible area. A written description of the condition of the unionid samples and their attached microbial mats was undertaken. Samples were also photographed. The identity of the unionid bivalves was confirmed by reference to Clarke (1981). Details of the microbial mat were compared with Huynh and Serediak (2006). In the absence of access to appropriate facilities to conduct the preparation and detailed systematic identification of microscopic epiphyte samples, we were limited to a visual description of samples at the field site.

RESULTS

Five live individuals of the unionid bivalve species *Elliptio complanata* were seen to host a bright green microbial mat (Figure 5.1). Substantial growths of microbial mat were observed along the dorsal region of the exterior of the basibiont host shells. The thickness of the microbial mat varied between samples, as well as spatially across the shell surface of individual bivalves. In all instances, growth was most pronounced in the posterior region of the shell, where it exceeded a centimetre in thickness. In addition, the microbial mat extended anteriorly through to cover the umbonal region of some specimens. Away from the shell posterior, the mat growth was generally several millimetres to a centimetre thick. As can be clearly seen in the figure, growth of the microbial mat was confined to physiologically inert regions of the shell surface, with protrusion of siphons and the foot remaining unimpeded. Similarly, the flexibility of the mat allowed

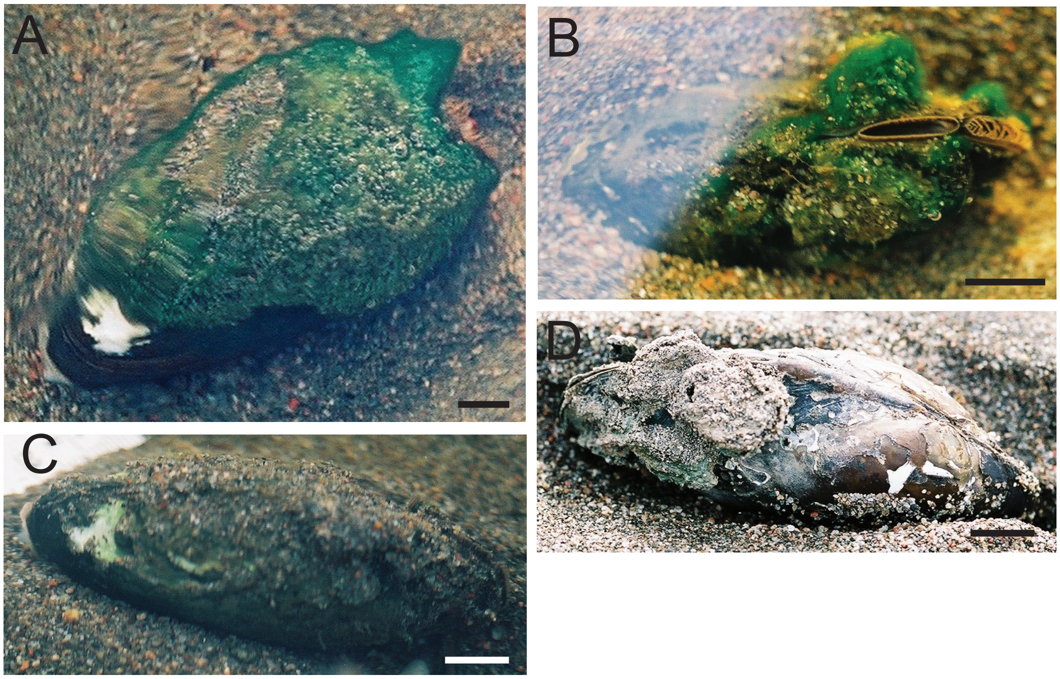

Figure 5.1. A-D) Macroscopic microbial mats were observed living epibiotically on several live individuals of the unionid bivalve *Elliptio complanata*. In all observed examples, microbial mat growth occurred in the dorsal region, being greatest at the posterior of the bivalve and extending to cover the etched umbonal region in some individuals. The live microbial mat was seen to be actively photosynthesising, as indicated by the presence of gas bubbles on its surface **(A and B)**. As is often the case in microbial mats occurring on fixed substrates, these microbial mats were also notable as effective sediment traps **(A-C)**. Although the live microbial mat was found to be extremely friable when handled, remnants of dead microbial mat nevertheless continued to adhere to some bivalves **(C and D)**. Scale bars = 1 cm.

normal operation of the hinge where growth had occurred along the dorsal margin of the bivalve. When handled, the live microbial mat readily disaggregated, however, despite this apparent fragility, two bivalve specimens were seen to have dead microbial mat still adhering to their shell. Portions of mat removed during handling displayed poor cohesion and revealed no apparent fibrous component or regular internal structure. Silt and sand sediment matching the local riverbed substrate were also noted, trapped within the mat, as seen in figure 5.1. The behaviour and condition of the basibiont unionids appeared similar to clean shelled individuals also observed at the same locality and there was no evidence to suggest their health had been impaired either prior to or following the growth of the mats.

DISCUSSION

Sedentary or sessile organisms inhabiting the aquatic realm may accrue a number of survival advantages by exploiting solid surfaces (see for example Wahl, 2010 for a recent review). As a consequence of this adaptive pressure, substrates that are freshly exposed within, or introduced into aquatic settings, are rapidly colonised. By way of illustration, in marine settings the creation of a community that included cyanobacteria and diatoms occurred within three weeks of a substrate being made available (Wahl and Olaf, 1999). The prevalence, and indeed predominance of benthic ‘algae’ within epibiotic communities in temperate and boreal marine settings is noted by Jackson (1977) and references therein. Initial colonisation rapidly results in the growth of adherent biofilms (see Romaní, 2010 and references therein). In many instances, more complex fouling communities will then develop. Such communities may be represented by, or at least include, microbial mats. The ecology of microbial mats is reviewed in Stal (2000). He provides a broad definition for microbial mats as being “multilayered microbial communities growing on sediments in a variety of different environments”. Microbial mats may be formed by components that include cyanobacteria, chlorophytes and diatoms, all of which occur in freshwater settings (Dodds and Whiles, 2010). The structure of typical cyanobacterial mats often includes the presence of a film of diatoms overlying the top of the mat, as well as a layer of sand or sediment. Scanning electron microscopy examination of the surfaces of dead empty unionid shells collected at our study site revealed the presence of biofilm growths, with both extracellular polymeric substances (EPS) and diatom communities (described and reviewed in Lawfield et al. in press). It is speculated that these biofilm components may also have formed a foundation for the microbial mats described here, which, based on their colour, morphology and textural properties, are likely dominated by either cyanobacteria or chlorophytes, or a combination of the two taxa.

The many and varied costs and benefits that may result for both basibiont host and any potential epibionts are outlined in Wahl's (1989) review of marine epibiosis. Many of the factors he describes for the marine realm should presumably be equally applicable in freshwater settings. One of his main conclusions is that basibionts generally accrue a net cost from hosting epibionts, but that this will be tolerated provided the benefits of investing energy in somatic growth outweigh the combination of energetic costs associated with investing in defensive measures and the potential for energetic saving that may accrue from possessing such defences. Few of the potential benefits to basibiont organisms that occur as a result of attached epibionts as listed by Wahl (1989) are likely to present a significant advantage to unionid populations. There is some potential for protection from predation via camouflaging, however the significance of predation on unionids represents another area that has been relatively poorly documented in the literature. Some published studies have addressed predation upon adult unionid populations, for which native (North America) and introduced (Europe) muskrats are considered to be the principle predators (e.g. Bauer, 2001, Zahner-Meike and Hanson, 2001) together with other mammals such as otter and raccoon (e.g. Strayer, 2008). A wider range of predatory fauna, including birds, fish and turtles are reported in Strayer (2008) and Walker et al., (2001) and references therein. The effect of muskrat predation may be locally of sufficiently high intensity in the nearshore areas of large waterbodies to "largely eliminate mussels" e.g. Zahner-Meike and Hanson (2001) and references therein. Zahner-Meike and Hanson (2001) also report clear trends of preferential predation on particular size cohorts, which varied with the prey species being targeted, larger (>90mm) *Pyganodon cataracta* were selected in contrast to some other, generally thicker shelled, species where smaller individuals were favoured. No studies directly address predation on *Elliptio*, however it might be considered to fall within the realm of the thicker shelled faunas. Of those negative selective factors of most relevance to the bivalve basibionts in the present study are the energetic costs associated with the additional weight burden during movement. The greater surface area presented to the potentially dislodging water current is unlikely to be a significant factor given the mass of the bivalves relative to the rivers velocity. The epibiont may gain some benefit from carbon dioxide and other excretory waste products derived from the bivalve, possibly including nitrogen (e.g. Vaughn and Hakenkamp, 2001 and references therein), in addition to the advantages that accrue to any organism exploiting a hard substrate in a water medium (e.g. Wahl, 2010). Additionally, of potential relevance to our discussion on the ecological significance of the association, cyanobacteria have been reported in symbiotic interaction contributing metabolic products (nitrogen or carbon) to their hosts e.g. Adams, (2000), although such interactions have not been reported to occur with either marine or freshwater molluscs.

In the case of unionid populations, similar associations to ours *may* have been noted previously, however beyond a cursory mention no further details have been provided. Spooner (2002) for example noted that unionid bivalves may be host to epibiotic 'algae' which favor live unionids for their nutrient excretion, whilst

Vaughn et al., 2002 and 2008 record ‘river weed’ and unspecified ‘periphyton’ growing on unionids. In addition, Tribollet et al., 2008 report the presence of the euendolithic cyanobacteria *Plectonema terebrans* giving rise to microboring, although these appear to be smaller growths than those encountered in our study.

On balance, the association we document is probably of a broadly neutralistic or mutualistic nature. Given the absence of any epibionts on the majority of bivalves observed at the site, the association between epibiont and basibiont described here is evidently a facultative one. Studies conducted in marine settings suggest that epibionts are generally not substrate specific (Wahl and Olaf, 1999), although ‘algal’ growth was found by Cox (1988) to be strongly dependant on local conditions. Despite the locally sandy substrate evidently proving unsuitable for microbial mat growth, similar growths were also noted on coarser grained pebble and cobble substrates encountered at greater river depths while approaching the study site.

Unionids possess a layered shell structure (e.g. Taylor et al., 1969), with an external periostracum (visible as darker regions of shell in figure 5.1) overlying a series of predominantly aragonitic interior layers (visible as lighter regions of shell in figure 5.1). The periostracum is thought to provide some degree of protection from physical and chemical damage to the shell. The taphonomic processes that may affect unionid shells are described and reviewed chapter three and references therein. Microbial mats may have an impact on the chemistry within their immediate vicinity, for example displaying rates of photosynthesis sufficiently high to cause local supersaturation and oxygen bubble formation (as clearly illustrated in figure 5.1). Although microbial mats are typically of alkaline pH, it has also been observed that the extracellular polymeric substances that they often contain may inhibit calcification (e.g. Stal, 2000, Dodds and Whiles, 2010) and elsewhere microbial mats and biofilms have been cited as agents of microbiocorrosion (Verrecchia et al., 2003, Ortega-Morales et al., 2004 and Tribollet et al., 2011). However, despite growth of the mat being observed to occur on both the periostracum and aragonitic regions of the shells, there appeared to be little difference between the loss of periostracum and degradation of shell material noted in basibiont shells versus those without any epibiont population. Thus, whether the microbial mat growths could have a significant impact on the deterioration of the shell exterior remains a matter of conjecture and to unequivocally answer this question would require further study.

Several authors have noted chemical (Bers et al., 2006) and physical (Scardino et al., 2003, Bers and Wahl, 2004, Bers et al., 2010 and references therein) adaptations of the periostracum that inhibit biofouling in a number of marine bivalve fauna. If any such adaptations are present in unionid bivalves they are evidently ineffective in the examples presented here. It is therefore curious that a greater degree of epibiosis has not been more widely reported and this apparent absence must be accounted for. One behavioural mechanism that has been noted to inhibit more widespread biofouling on unionids is burrowing (e.g. Nichols, 2002), with passage through the substrate sloughing off attached organisms. The burrowing behaviour of unionid bivalves is reviewed in detail by Lawfield and Pickerill (2006), and references therein and also in chapter six. Depending on the unionids species, the season and location, an endobenthic or epibenthic mode of life may be adopted, with preference for full burial being often accompanying harsher environmental conditions, such as those encountered during winter. Generally, the life orientation of unionid bivalves appears to be relatively poorly documented, and we were unable to locate accounts or figures illustrating this for *Elliptio* populations in general. Published studies of other genera suggest that adult unionids are predominantly epibenthic and burrowing is generally associated with finer sediments than those encountered at our study site (Amyot and Downing, 1997, Nichols, 1997). The presence of microbial mat growths on our study samples would add further evidence to suggest that burrowing behaviour is either infrequent in the studied specimens or when undertaken leaves at least a third or more of the shell exposed above the substrate surface. Such a conclusion is entirely consistent with the nature of the predominantly surficial tracks described in Lawfield and Pickerill (2006). Given these observations, it is uncertain whether the microbial mats described in our study are a permanent fixture on the shell surface, although this is probably the case. It is however entirely possible that their growth could also be of a seasonal or episodic nature, particularly given the potential for rapid growth exhibited by the organisms in question.

SUMMARY

1) Observations undertaken on live unionid bivalves in the Saint John River, New Brunswick, Canada, revealed their exploitation as a substrate for epibionts. Substantial growths of a bright green microbial mat were observed along the dorsal region of the basibiont host *Elliptio complanata*.

2) Growth of the microbial mat was most pronounced in the posterior of the shell, extending to the umbonal region.

3) The bivalves hinge operation and protrusion of soft tissue, including both the foot and siphons, remained unimpeded.

4) Although unionids are capable of burrowing, the presence of microbial mat growths suggests this behaviour is infrequent, or at least restricted in the observed specimens.

5) This freshwater microbial mat structure is assumed to be dominated by cyanobacteria, chlorophytes, or some combination of the two taxa.

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CHAPTER 6

FRESHWATER UNIONID BIVALVE SHELLS AS SUBSTRATA FOR TRICHOPTERA ATTACHMENT[1]

INTRODUCTION

Unionid bivalves (freshwater mussels) occupy an important ecological position within both river and lake environments, as well as being found preserved in their associated sedimentary deposits. Although they occur exclusively in freshwater settings, unionids embody most of the characteristics shared by the majority of marine basibionts (defined by Wahl, 1989 as organisms that provide a substrate for populations of attached epibiotic organisms). Wahl and Olaf (1999), provide a list of the characteristics of ideal basibiont organisms, which include longevity, relatively large size, a slow moving epibenthic habit and "physiologically inactive body surface areas" (in this instance embodied by the shell exterior). Although epibiotic associations have previously been reported for unionids, most published works focus exclusively on the impact of invasive *Dreissena* (Zebra Mussel) infestations (see for example Nichols, 2002 and Hörmann and Maier, 2006 and references therein). As a result, there are few publications addressing the wider community of unionid hosted epibiota (e.g. Beckett et al., 1996; Spooner, 2002; Vaughn et al., 2002 and 2008; Tribollet et al., 2008). In marine faunas, many potential basibiont organisms employ chemical and physical mechanisms to inhibit the attachment of epibiota (Scardino et al., 2003; Bers and Wahl, 2004; Bers et al., 2006; Bers et al., 2010 and references therein). If

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[1] *A version of this paper has been submitted for publication. Lawfield, Gingras, Pemberton and Erickson. Palaios.*

present, these mechanisms have not been reported for unionids. We have observed concentrations of Trichoptera (caddisfly) attached to unionid shells in a modern river setting and details of this association are presented herein.

STUDY SITE AND METHODS

The study was conducted in a dam-controlled reach of the Saint John River, Fredericton, New Brunswick (45°58′ N, 066°42′ W). Observations were undertaken upon an assemblage of bivalve remains and associated epibionts revealed by a seasonal low water event at the end of summer, during September, that resulted in a one metre drop in water level. The samples were observed occurring on an exposed sandbar at the western end of Jewett Island, and in the adjacent shallow water at the edge of the river channel. The riverbed at the site where observations were made was predominantly sand, with an increasing proportion of gravel and cobbles observed with increasing water depth towards the centre of the channel. At this point in its course, the river has a wide channel and shows an anastomosing plan view morphology, with approximately 700 m total width of water divided between two or three channels that are separated by several small islands, the main channel being 500 m across. As a consequence of its width and corresponding low levels of shading by vegetation, rocks on the riverbed are exposed to sunlight, thus favouring growth of periphyton such as diatoms that may be exploited by grazing organisms, including Trichoptera (e.g. Wiggins, 1996, 2004; Vineyard et al., 2005; Ames, 2009). There is relatively little vegetation in the channel and similarly, locally, low levels of visible detrital organic material are observed deposited on the riverbed, which is predominantly of clastic sediment. The rivers current is of moderate speed, reaching a maximum velocity of approximately 1.5 m/s at the centre of the channel and being lower towards the banks. Further details of the hydrology, climate and sedimentology are presented in Lawfield and Pickerill, 2006.

A 100 m stretch of sandbar and adjacent riverbank was measured, within which observations were conducted on all the unionid bivalve specimens observed on the recently subaerially exposed sediment, as well as below the newly established temporary water level to a depth of approximately 50 cm. This depth point was selected to enable easy observation of specimens and as demarcating a distinct increase in current velocity together with a correspondingly higher grain size of the riverbed sediment below this depth.

All accessible bivalve shells observed on the bank and in the shallow water were examined visually for the presence of epibionts, the identity, number and location of which were subsequently documented. A number of shells were collected and subjected to further examination with a scanning electron microscope (SEM).

The identity of the unionid bivalves was confirmed by reference to Clarke (1981). The taxonomy of case making forms of caddisflies was attributed to genus level on the basis of case morphology and in the example of *Helicopsyche* refined to species level through knowledge of geographical distribution, *H.borealis* being the only known species with a Canadian provenance (Wiggins, 1996).

Preparation for SEM imaging involved selection of unionid bivalve shell material with a taphonomic range from pristine to poorly preserved, to enable comparison between a variety of distinct shell surface textures. Selected samples were cut to a size appropriate for introduction to the SEM apparatus. This was undertaken by first cutting samples by hand with a hacksaw. A cross sectional view displaying the layered structure of the shell was subsequently created across one edge of the sample (Figure 6.1) by fracturing with pliers. The samples were then mounted on stubs using epoxy resin before being sputter coated with gold. Imaging was undertaken at 5.0 kV with a Jeol 6301F Field Emission scanning electron microscope.

RESULTS

Three species of unionid bivalves were encountered, they included *Lampsilis radiata radiata* and *Elliptio complanata* which together formed the majority of the assemblage, together with rarer examples of *Anodonta implicata*. The studied bivalves occurred as dead, empty, articulated shells. All observed bivalves displayed some degree of taphonomic decay, with a variable extent exhibited both between individuals and spatially across the shell surface within individual specimens. In all cases the umbo exhibited some degree of decay (Figure 6.1). The full range and significance of the taphonomic decay are described fully elsewhere (chapter three). Several types of freshwater epibiont populations were observed, with both microscopic and macroscopic examples represented, the main microscopic forms include diatoms, bacteria, and biofilms (Lawfield et al., in press). The main macroscopic forms were a photosynthetic biomat or microbial mat occurring on live individuals that is described elsewhere (chapter five), together with Trichoptera represented by final (fifth) instar larval or pupal stage case forms.

The Trichopteran assemblage (Figure 6.1) included abundant *Neophylax* sp. together with common *Goera* sp. and rare *Helicopsyche borealis*. The first two caddisfly genera possess linear case forms that are distinguishable by the presence

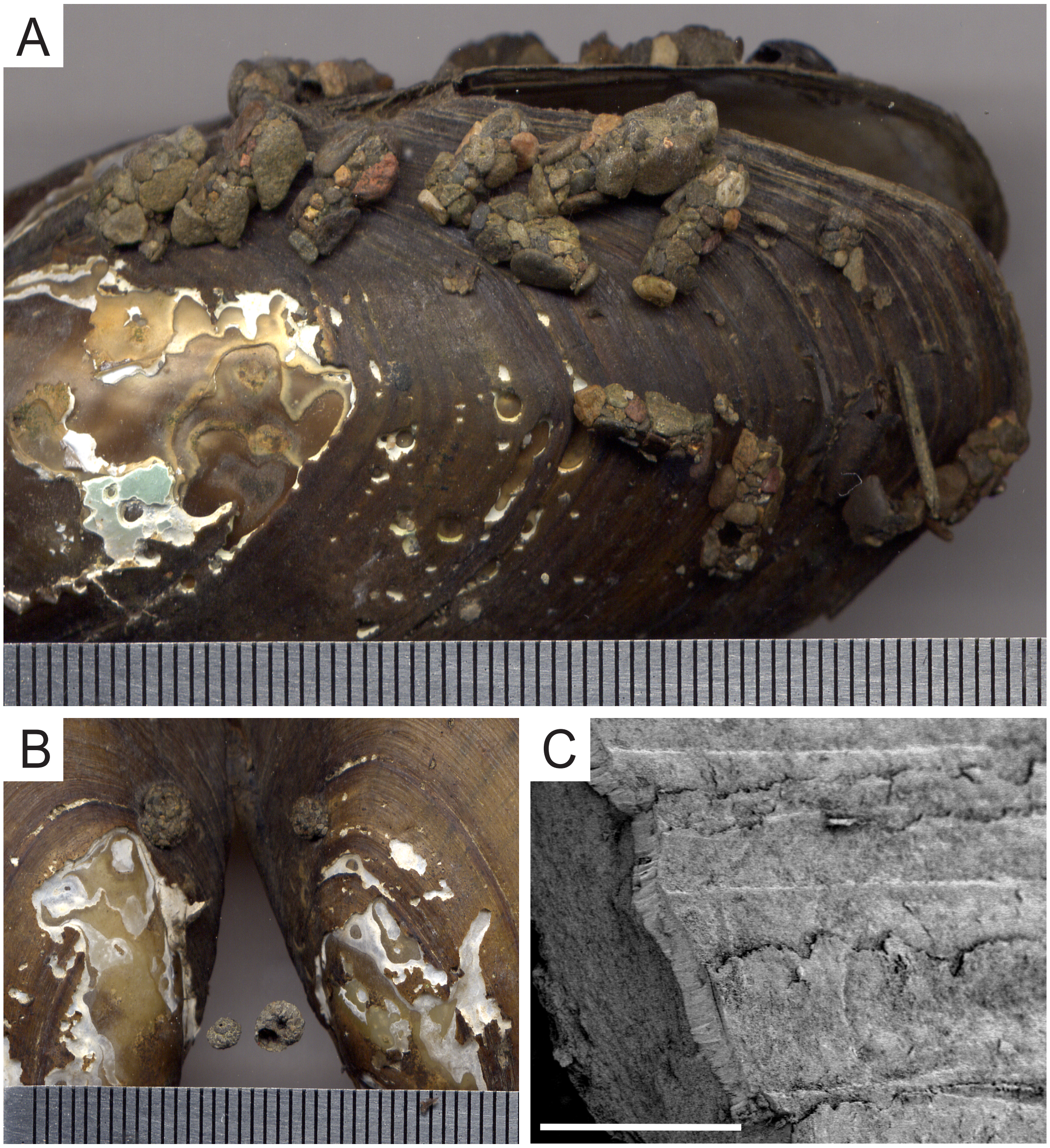

Figure 6.1. A and B) Trichoptera cases in either their final (fifth) instar larval or pupation developmental stages were observed attached preferentially to intact periostracum in the posterior region of the exterior of dead, empty, unionid shells, avoiding anterior regions, as well as the umbo and other areas of taphonomically degraded shell. The caddisfly case assemblage includes linear *Neophylax* sp. and *Goera* sp. (the latter distinguished by the presence of only two lateral ballast stones on each side and the marked symmetry of the size of stones on both sides) present on the unionid Anodonta implicata **A)**, as well as the spiral case form *Helicopsyche borealis*, on *Elliptio complanata* **B)**. Scales for A and B in mm. **C)** Scanning electron microscopy image of the unionid bivalve *E.complanata*, displaying the shell exterior in addition to a cross section (at left), illustrating the exterior organic conchiolin periostracum overlying the interior aragonitic prismatic and nacreous shell layers. Scale bar = 1 mm.

of multiple smaller lateral ballast stones on each side in specimens of *Neophylax* sp. or only two larger lateral ballast stones on each side of the case in examples of *Goera* sp. (Wiggins, 1996). *Helicopsyche* is readily differentiated by its distinctive helically coiled form (Wiggins, 1996).

Case attachment behaviour was observed on 29 % of individual unionid specimens (n = 35). The number of bivalve shells found to bear Trichopteran cases was distributed as follows: *Neophylax* sp. was present in monogeneric groupings on three shells, bearing respectively three, two and one individual pupal case (mean of two *Neophylax* cases per shell). Monogeneric *Goera* sp. was noted on three shells, each of which bore one individual case. Monospecific *Helicopsyche borealis* was present on three shells, bearing respectively two, one and one cases (mean of 1.3 per shell). In addition, a mixed assemblage that comprised thirteen *Neophylax* and four *Goera* cases was present on one individual bivalve.

No selective preference was noted for attachment to particular unionid species, however within individual bivalve shells, site selective attachment behaviour was noted to occur spatially across the shell surface. Unionid bivalves possess a distinctive and characteristic layered shell structure (Figures 6.1C and 6.2). Describing this from the outside, there are first encountered two thin outer layers, the outermost being of organically composed periostracum that overlies a prismatic crystalline aragonite layer. These in their turn overly a much thicker series of nacreous aragonite layers interspersed with irregularly spaced layers of organic material that together form the majority of the shell thickness (Taylor et al., 1969; Checa, 2000; Checa and Rodríguez Navaro, 2001). As noted elsewhere (e.g. chapter three), physical and chemical taphonomic processes serve to erode the shell, both within the lifetime of, and to a more significant degree following

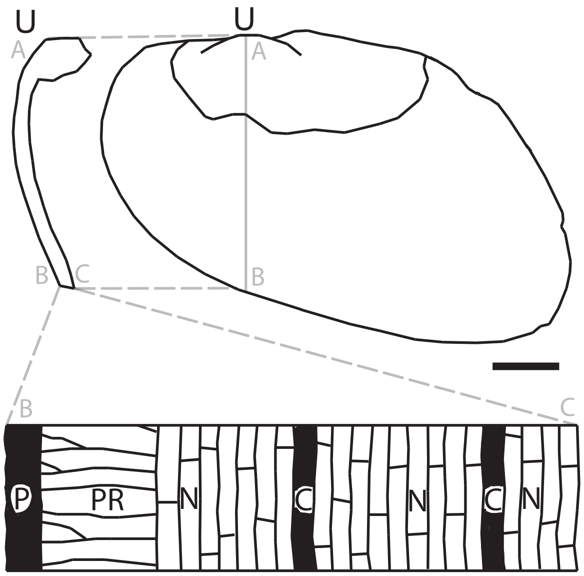

Figure 6.2. Diagrammatic cross section of unionid shell, indicating placement of shell layers. Upper images show the shape of the shell. **Top left)** Apical plane cross sectional view. **Top right)** Lateral view. Pale grey line A-B = line of section. U = umbo. Line surrounding umbonal region, shown in the top right figure approximately demarcates the umbonal area commonly affected by loss of the periostracum layer, with predominantly intact periostracum occurring across the rest of the shell surface. Scale bar for both images = 1cm. **Bottom)** Cross sectional view penetrating shell. B-C = line of section. Black regions = areas of organic proteinaceous conchiolin. White areas = crystalline aragonite. P = periostracum, composed of conchiolin. PR = prismatic aragonite layer. N = nacreous aragonitic layers. C = conchiolin. Lower cross sectional view is a representative image, not to scale and doesn't illustrate the prolific number of nacre layers present, which typically occur in numbers an order of magnitude higher than those depicted here. From Lawfield et al., in press.

the death of the bivalves themselves, resulting in the exposure of these multiple shell layers. Any prospective epibiont therefore has a range of potential surface attachment points from which to choose, each with widely differing textural and chemical properties.

Our observations indicate that both the crystalline aragonite and organic layers exposed within damaged regions of the molluscan shell were universally avoided.

Trichopteran attachment was instead confined to areas of the intact unionid shell periostracum (Figure 6.1). The spatial distribution across the shell also showed a distinctive preferential spatial grouping, with all attached caddisfly retreats being found posterior to the umbonal area, the periostracum perhaps being selected for it's rough surface texture in contrast to the comparatively smoother aragonitic and interior organic areas.

During handling of the samples some pupation cases were dislodged, however despite intensive examination of the shell material exposed underneath the former attachment points of these cases, no damage was noted to the underlying shell.

DISCUSSION

Sedentary or sessile organisms inhabiting the aquatic realm may accrue a number of survival advantages by exploiting solid surfaces (see Wahl, 2010 for recent review), such exploitation may include the use of an organism as a substrate, where the attached organism is termed an epibiont and the substrate organism is termed a basibiont. The many and varied benefits and costs that may result for both basibiont host and any potential attached epibionts are outlined in Wahl's (1989) review of marine epibiosis. Many of the factors he describes for the marine realm should presumably be equally applicable in freshwater settings. In the case of basibiont organisms, Wahl (1989) emphasises the complex interplay between investing energy in somatic growth weighed against the costs and potential benefits of investing in defensive measures. As a result, although basibionts generally accrue a net cost from hosting epibionts, this will often be tolerated as a result of the potentially significant energetic expenditure required to inhibit their attachment. Several authors have noted chemical (Bers et al., 2006) and physical

(Scardino et al., 2003; Bers and Wahl, 2004; Bers et al., 2010 and references therein) adaptations of the periostracum that inhibit biofouling in a number of marine bivalve fauna. Our observations suggest that physical barriers are absent from the periostracum of unionids. Furthermore, if any such adaptations (either physical or chemical) are present in unionid bivalves they are evidently ineffective in the examples presented here.

Hershey and Lamberti, (2001) note that “at a local scale in stream ecosystems, substrate and current velocity are probably the most important physical factors determining the community structure of aquatic insects.” They also emphasise that “stream insects spend most of their lives attached to substrates.” Other factors that are noted as commonly influencing insect distribution include oxygenation and temperature. Wallace and Anderson (1996) remark on the generally unfavourable nature of river and stream sand substrates for exploitation by aquatic insects, owing to an absence of attachment sites and a general paucity of food resources, both of which would potentially make unionids an attractive substrate where these occur on sand riverbeds. Jackson (1977) notes that small hard substrates, such as shells or stones, that are susceptible to burial or overturning, are predominantly colonised by solitary organisms. In freshwater settings this bias is presumably further influenced by the preponderance of solitary organisms and this is supported by observations on freshwater epibiota assemblages undertaken by Beckett et al., (1996). The use of unionid bivalves as a substrate by Trichoptera has previously been noted by Beckett et al., (1996), although they don’t provide a detailed description of the attachment patterns in their paper. They were led to conclude that the selection of unionids as a substrate for attachment appeared to be purely a function of the shells providing an available hard substrate and that the attachment behaviour could not be ascribed to some unique property of, or association with, the unionids themselves.

Most studies addressing epibiotic interactions emphasise marine organisms at the expense of neglecting freshwater associations. The various attachment strategies adopted by marine epibionts are reviewed in Bromley and Heinberg (2006) and to date there has been no comparable review for freshwater forms, although there is presumably overlap between the two aquatic realms. Attachment of the Trichoptera cases occurs via silk threads that are also employed to bind the components of the Trichoptera cases themselves during their construction (Wiggins, 2004). The silk threads appear to create a relatively robust attachment, despite leaving the underlying shell material physically unaltered.

In our assemblage the available substrate comprises unionid bivalve shells. It is worth noting that the life orientation of the unionid bivalves (Figure 6.3) obviously exerts a profound influence on the regions of shell that remain above the sediment-water interface and are thus available for colonisation by any potential epibiota. Some discussion of life position is presented in Lawfield and Pickerill (2006) and references therein, including Di Maio and Corkum, (1997). Although McMahon and Bogan, (2001) note that “pedal surface locomotion is reduced or lost in most adult unionoideans” that is not supported by our observations. Lawfield and Pickerill, 2006 noted that populations of live individual unionids at the study site were free living and generally horizontally aligned, with the majority of the shell, including the full umbonal region and the upper posterior portion of the shell proud of the substrate. Some examples were fully buried with a vertical orientation, but this alignment was adopted as a response to falling water levels. A review of the sparse literature on this subject suggests that partial burial is common, but the figured examples we encountered show the umbonal region being clear of the sediment and

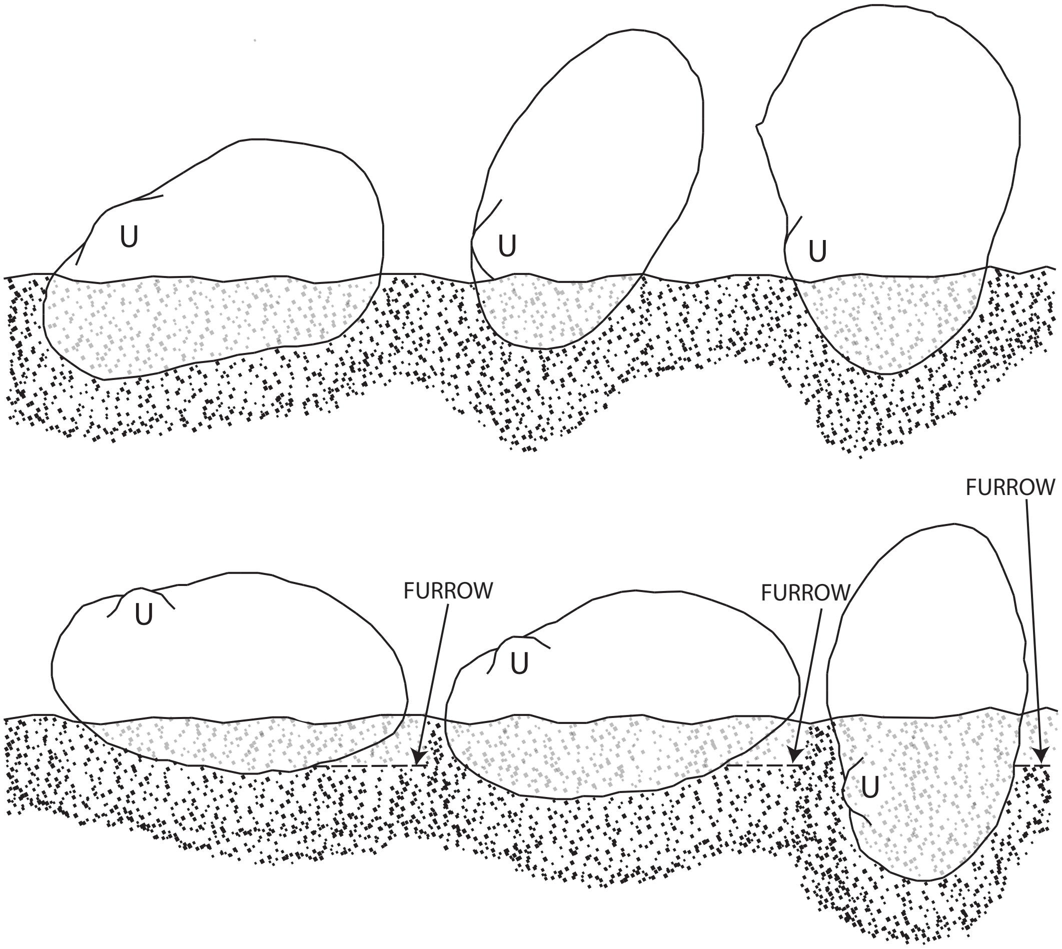

Figure 6.3. Range of life orientations exhibited by unionid bivalves. **Top row)** Literature examples, shown in simplified and modified form. **Top left)** *Lampsilis siliquoidea*, after Pennak 1989, itself modified from Baker, 1928. **Top centre)** *Lampsilis perovalis*, after Haag et al., 1995. **Top right)** *Lampsilis ovata*, after McMahon and Bogan, 2001. **Lower row)** Representative variation in orientations of live unionids observed during our field data collection. Bivalves with orientations the same as those in the lower left and middle images included examples hosting microbial mat growths (see chapter five), the extent of which suggests that deeper burial does not occur in these bivalves. The orientation depicted in the lower right appears to be atypical for the studied assemblage having clearly been arrested in place during emersion as the water level dropped below the sediment surface. Although we have not indicated the position of soft tissue, in all examples except that figured at lower right, bivalves were noted to be gaping and with soft tissue including siphons protruding. For all images: Dotted pattern = sand substrate. Irregular, continuous horizontal line above the dotted pattern = substrate-water interface. Inferred margins of substrate concealed portions of the shell are shown. Dashed lines = position of furrows created during movement of the bivalves (for a more detailed

description of these trace features refer to Lawfield and Pickerill, 2006 and chapter two).

thus available for colonisation e.g. Pennak (1989). Our field observations and a review of the available literature suggest that the umbonal region, as well as other regions of shell surface denuded of periostracum, would often be available for unimpeded epibiont colonisation.

Caddisflies (Order Trichoptera) are a diverse (approx 10,000 species), prolific and globally distributed order of insects (Wiggins, 2004). Caddisflies have a long fossil record, with a stem group dating to the Triassic (Wiggins, 2004) and modern families being recognisable from the Jurassic onwards (Grimaldi and Engel, 2005). The earliest known examples of preserved cases are of Jurassic age (reviewed in Grimaldi and Engel, 2005). Preserved cases may occur in locally prolific concentrations, as is the case in the Miocene indusial limestones of France, where they represent a dominant component of the rock (Lyell, 1837; Scudder, 1890; and see also review in Hugueney et al., 1990). Preserved caddisfly cases have historically been taxonomically placed alongside body fossils, being referred to taxonomically by the name of the constructing organism, or as Indusia if the identity of the responsible Trichoptera taxa cannot be determined (e.g. Scudder, 1878; Scudder, 1890; Cockerell, 1925; Hugueney et al., 1990), or more latterly as trace fossils. A classification scheme based on case material and construction method has also been erected (e.g. Vialov and Sukatsheva, 1976). Our assemblage comprises portable-case-making forms, a mode of life for which representative fossilised cases are known from the mid-Jurassic onwards, with extant families beginning in the early Cretaceous (Wiggins, 2004). Our observations demonstrate that unionids act as hosts to attached Trichoptera, an association that has been documented from a fossilised Palaeocene example

(Erickson, 1983), but based on the age ranges of the involved taxa may also have existed during the Mesozoic.

Several factors complicate the quantification of observations relating to retreat attachment upon unionid substrates. Attachment of cases is only associated with particular stages in the Trichoptera life cycle, with the final (fifth for most genera) larval instar modifying it's larval case or constructing a pupal case which is attached to the substrate before the ends of the case are sealed. The caddisfly then progresses through the prepupal, pharate pupal and pupal phases within the case, before developing into a pharate adult. Once this stage is reached the caddisfly cuts open the anterior end of the pupal case from which it subsequently emerges (e.g. Wiggins, 1996, 2004). These phases in the Trichoptera life cycle occur within a narrow seasonal range, cued by environmental temperature changes. Although not previously exploited, the seasonality of these processes may provide valuable palaeoecological information relating to the age of any preserved Trichoptera-unionid associations. Burrowing activity by unionids that might remove attached Trichoptera also has a seasonal component, often being initiated to avoid harsh winter conditions (see review and references in Lawfield and Pickerill, 2006). Local variation in substrate, water depth and current velocity may also all play a significant role in determining whether a potential attachment site will be selected or avoided by Trichoptera (Wiggins, 2004) and similarly may also affect unionid distributions. These environmental preferences will obviously play a significant role in influencing the distribution of locations where epibiotic associations might reasonably be expected to occur. Another complicating factor in study of the preservation potential of such associations is the complete dearth of knowledge relating to the taphonomic processes acting upon caddisfly cases. No actualistic studies are available in the literature and details of the processes that act to destroy cases and the timeframe over which they operate are not documented. Similarly, the robustness and duration of attachment to the substrate of vacated Trichoptera cases is also a matter of speculation. There is evidently substantial scope for additional future study to investigate this further.

In their larval stage, caddisflies inhabit a diverse range of aquatic habitats. The presence of taxon specific preferences for particular environmental parameters enables them to serve as important ecological indicators within freshwater ecosystems (e.g. Wiggins, 2004; Ames, 2009). The majority of taxa occur in lotic (moving water) settings, where they generally adopt an epibenthic habit. The association we observe includes families of Trichoptera that display a preference for and are for the most part in fact confined to lotic (running water of streams and rivers) habitats. Members of the Helicopsychidae are also known to occur in higher energy, rocky, wave-washed littoral zones of lakes (Wiggins, 2004). The Trichoptera cases we observed all fall within the Suborder Inegripalpia, which groups the portable-case-making taxa and separates them from the closed-cocoon-making and fixed-retreat-making taxa which are ascribed to the Spicipalpia and Annulipalpia respectively. In the majority of case making taxa, including those in our assemblage, pupation occurs within the larval cases that are initially constructed and employed in a mobile form during foraging behaviour from the first larval instar onwards (e.g. Wiggins, 2004). Linear case making caddisfly forms belonging to the families Uenoidae and Goeridae are generally considered to be confined to habitats with hard substrates dominated by large sized rocks, which may themselves provide an attachment point for caddisfly cases during pupation. In the example of *Neophylax* “larvae grow during autumn and winter months; in spring and early summer final instars fasten their cases to rocks and seal off the openings” (Wiggins, 1996). The presence of large ballast stones and cap stones at the end of each of the cases in our assemblage indicates that the Trichoptera we observed were in the final (fifth) larval instar and/or the pupation stage (Wiggins, 1996, 2004; Ames, 2009), which in the case of *Neophlax* may span a period of several weeks (Wiggins, 1977). Wiggins (1996) also reports a period of up to six months spent in prepupal diapause for *Neophylax* following sealing of the pupal enclosure. Adult *Neophylax* emerge during the late summer and autumn (Wiggins, 1996). Larvae of *Neophylax* pupate in aggregations (Wiggins, 2004), and although apparently not previously reported for *Goera*, our assemblage suggests that the same behaviour appears to be indicated for that Genus. The lifecycle of most caddisflies is completed in one year, however members of the Goeridae are unusual in requiring more than one year (Wiggins, 2004). Such observations on seasonal events in the lifecycle of Trichoptera suggest that if larval cases are encountered in the rock record that they may serve as useful indicators for constraining seasonal deposition. Other information relating to climate might be derived in a similar fashion to the existing use of caddisfly body fossils in palaeoecology (e.g. Williams, 1988).

The use of shells as a substrate was observed in situations where an otherwise essentially planar sand substrate was broken by protruding shells embedded in, or overlying the sand. At this locality, larger cobble substrates were generally confined to deeper, higher velocity waters towards the centre of the channel. Since Trichoptera distribution is influenced by factors that include substrate grain size and water velocity, the shells may act as a substitute for appropriately large grain size rocky substrates, or alternatively, our site may represent a location peripheral to the main population. The gregarious clustering of the linear case making forms, that were encountered in either monogeneric or mixed genera assemblages are consistent with Wiggins (2004) observation that clustering occurs by caddisflies larvae actively selecting pupation attachment sites adjacent to previously emplaced individuals that possess cases of similar surface roughness.

All the observed case making caddisfly forms feed as herbivores and detritivores, grazing on periphyton, including diatoms, and also on detrital particulate organic material (Wiggins, 1996, 2004; Ames, 2009). Several families of Trichoptera, including those represented in our present study (Goeridae, Uenoidae and Helicopsychidae) have adopted a scraping functional feeding group (*sensu* Merritt and Cummins, 1996). Diatoms are described as a "mainstay" of modern aquatic insect scrapers, with cyanobacteria being inferred as a food source prior to the early Cretaceous arrival of diatoms in freshwater settings (Wiggins, 2004). In more detail, larvae of *Helicopsyche borealis* are reported as feeding on "diatoms and detritus" (e.g. Williams et al., 1983), or more broadly on "algal, detrital, and animal materials." (e.g. Coffman et al., 1971 and Mecom 1972 cited in Wiggins 1996) or grazing "periphyton and fine organic particles from rocks" (Wiggins, 2004) and for *Goera* "scraping periphytic algae and fine detrital particles from exposed surfaces of larger rocks" (e.g. Coffman et al., 1971 cited in Wiggins 1996). Members of the genus *Neophylax* "feed by grazing diatoms and fine organic particles from rocks" (Wiggins 1996). SEM observation revealed localised diatom populations on a number of dead unionid shells (Lawfield et al., in press), however the distribution of these components on live individuals has not been documented and it is uncertain whether these are exploited as a food source by caddisflies and whether they influence the chosen attachment sites.
In addition, the possibility that the observed site selection might be based on a selective preference for regions of shell with enriched food sources would appear unlikely given that regions of biofilm hosting components including extracellular polymeric substances (EPS), as well as diatoms and other microorganisms

are observed occurring on exposed interior organic layers within the nacreous aragonite portion of the shell structure (Lawfield et al., in press).

The association we observed between Trichoptera and unionid bivalve substrates is apparently long established in the fossil record, dating back to at least the Palaeocene. A preserved example exhibiting seventeen caddisfly cases, occurring on a single, articulated unionid bivalve specimen, interpreted as being in life position has been described from the Palaeocene of North Dakota, USA (Erickson, 1983). Although in the sample Erickson describes, accurate details of the original extent of periostracum have been lost as a result of the mode of preservation in the specimen, we note that the umbonal region, which through taphonomic processes is often devoid of periostracum from a relatively early point in the growth of unionid bivalves, has been avoided. In the study by Erickson (1983) the restricted caddisfly case distribution is interpreted as being a consequence of the unionid adopting a semi-infaunal life position, precluding attachment except in the posterior regions of the shell. A thin outer layer is visible across the shell surface in Erickson, 1983 (his figure 2) that crosses growth lines and may correspond to the original position of the periostracum. The preserved caddisfly cases all occur on top of this layer. To the best of our knowledge this is the only example of an association between unionids and Trichoptera being reported in the literature as having been preserved in the sedimentary rock record. Casual observations by Dr Mark Erickson, conducted over a career spanning forty years and including examination of tens of thousands of modern freshwater mussels suggest that caddisflies frequently occur on both live and dead unionids (Dr M.J. Erickson, personal communication, 2013). Although these observations relate primarily to lotic river settings, Dr Erickson also reports attached Trichoptera occuring on unionids in higher energy areas of lakes, including the “rocky shore high energy zone of Missisquoi Bay of northern Lake Champlain in Vermont" (Dr M.J. Erickson, personal communication, 2013). The seasonality of observations, local variations in environmental conditions and the fact that such observations were not conducted systematically prevent accurate quantification of the proportion of affected shells. On occasion the dead shells had their interior surfaces exploited as a substrate (Dr M.J. Erickson, personal communication, 2013), indicating that Trichoptera attachment to nacreous shell is indeed possible, but observation of attachment to the exterior shell surfaces correspond with our observation that attachment occurs preferentially upon areas of periostracum.

Given the apparent abundance of modern occurrences of unionid-Trichoptera associations it is quite surprising that they have not been more widely studied or reported from either modern localities or examples in the sedimentary record. With comparatively common preservation of both unionids and Trichoptera in the fossil record when occurring in isolation, the apparent rarity of preserved associations between unionids and attached Trichoptera (or indeed any epibiota) deservers further investigation and we hope that more examples may come to light in the future. Given our observations on case attachment, it is likely that the low probability of preserving periostracum is a significant factor in this (Harper, 1997). It is however disappointing to note that the trend to overlook epibionts noted by Lescinsky (1996) appears to remain very much in place, in both biological and geological studies.

SUMMARY

1) Epibiotic associations in freshwater settings remain a largely neglected area of study. This applies both to the literature describing modern environments, as well

as to those river and lake deposits preserved in the sedimentary record.

2) Examination of unionid specimens from the Saint John River, Fredericton, New Brunswick, Canada revealed their colonisation by attached Trichoptera (caddisfly) cases, in either their final (fifth) instar larval or pupal developmental stages. The Trichopteran assemblage, identified from case morphology, refined by knowledge of geographical ranges, included abundant *Neophylax* sp. together with common *Goera* sp. and rarer *Helicopsyche borealis*.

3) In our samples, there appears to be evidence for site selective attachment of epibiota, apparently influenced by substrate composition and structure (in this case governed by the unionid bivalves multiple layered shell structure). For all caddis genera, site selective attachment occurred upon the shell surfaces. Crystalline aragonite and organic layers exposed within taphonomically damaged regions of molluscan shell were avoided. Attachment was instead confined to areas of intact periostracum posterior to the umbonal region of the shell, the periostracum perhaps being selected for its rough surface texture in comparison to aragonitic layers.

4) Many marine basibiont faunas, including bivalve molluscs, are known to employ a range of chemical and physical deterrents to minimise the incidence of epibiotic attachment, with its attendant detrimental impacts. Observations undertaken on the unionid bivalve shells using scanning electron microscopy suggest that physical barriers to attachment are absent within these faunas, and evidently, any chemical barriers, if present, are largely ineffective.

5) Although the fragility of the Trichoptera cases in this association would tend to

inhibit preservation in the rock record, they occur commonly in the present day, are known to exist from a preserved Palaeocene example and include components that are individually widespread in the Mesozoic.

6) Examples of Trichoptera cases dislodged during handling of specimens revealed no damage to the underlying shell. There is however currently no literature available addressing the robustness of Trichoptera case attachment to substrates and similarly no published studies have been undertaken indicating the diversity of taphonomic processes that Trichoptera cases are subjected to or the rapidity with which these might occur.

7) Knowledge of the seasonal life cycle of modern Trichoptera and the rapidity of decay processes that operate on their cases has the potential to provide detailed information regarding sedimentary deposits, for example concerning the timing and seasonality of episodic depositional events.

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CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

INTRODUCTION

This study undertook to document observations relating to the ichnology, taphonomy and epibiota of freshwater molluscan assemblages, with an emphasis being placed on unionid bivalve faunas. The diversity of soft sediment and shell boring trace forms, the nature of taphonomic processes in operation and the range and distribution of attached epibiota were all examined and catalogued.

Although freshwater ichnology has been approached within the ichnofacies model, with several prominent published studies in place that generally emphasise lacustrine depositional settings (e.g. Buatois and Mángano, 2004; Melchor et al. 2012 and references therein), the full complexity of freshwater trace assemblages has not been addressed and comprehensive study of ichnology in river settings remains comparatively neglected (e.g. Melchor et al. 2012 and references therein). This is in spite of the ecological significance of river environments (e.g. Thorp and Covich, 2001) and their importance as pathways for the evolutionary invasion of terrestrial environments by animals (e.g. Thorp and Covich, 2001), as conduits for basinward sediment transport and as sedimentary environments in their own right (e.g. Galloway and Hobday, 1996; Miall, 2010). In addition, although the ecological significance of unionid faunas has been recognised (e.g. Strayer, 2008; Cummings and Graf, 2010), the contention of Lescinsky (1996) that epibiont populations in marine settings are frequently ignored is even more applicable in the freshwater realm. There has been little detailed study of the relationship between unionids and epibionts in the context of pristine native epibiotic associations and the scope for their study is now being increasingly threatened as invasive epibiotic fouling bivalves (e.g. Cummings and Graf, 2010 and references therein) displace existing native biotas. These and other stressors are increasingly threatening unionid populations.

The study presented herein sought to highlight the significance of freshwater molluscs and in particular unionid bivalves as agents of trace formation in soft sediment ichnology. Additionally, the interplay of the varied taphonomic decay processes that operate upon the shells of unionid bivalves and the significant influence of the layered shell structure upon these were examined. In addition, the study emphasis the ecological significance of unionid bivalves, with close relationships being demonstrated between unionids and a variety of organisms that exploit the unionids shell structure as a substrate. These result in a varied assemblage of microborings and epibiotic structures.

This study thus aimed to expand upon existing knowledge within three key areas:
1) Freshwater ichnology, including both soft sediment traces and hard substrate microboring assemblages.
2) Early stage taphonomic processes of freshwater unionid bivalve assemblages.
3) Freshwater epibiotic associations at both microscopic and macroscopic scales.

PRINCIPLE FINDINGS AND THEIR IMPLICATIONS

The following list presents a chapter by chapter summary of the major findings of the study, together with the most significant conclusions arising therefrom:

1) **Chapter two** presents an example of a soft sediment fluvial channel trace

suite demonstrating a wide diversity of trace morphologies, including varied, horizontally aligned furrowed, meandering, looping and spiral plan view locomotion/grazing traces. These traces occur together with almond shaped *Lockeia* like resting traces within a shifting sand softground substrate. Simple vertical burrowing to avoid emersion as a result of falling water levels is also recorded. These traces are all ascribed a molluscan origin and with confirmed identifications of named trace makers, the described assemblage represents a valuable addition to the descriptive literature on fluvial ichnological assemblages (e.g. Melchor et al., 2012). Although their fluvial channel setting would place this assemblage within the *Scoyenia* ichnofacies, there is some overlap between features of this assemblage and those ascribed to the *Mermia* ichnofacies that is commonly assigned to trace assemblages with a lacustrine or floodplain pond origin. This suggests that there is scope for reevaluation and refinement of established freshwater ichnofacies models. It also highlights the importance of incorporating detailed simultaneous observation of both ichnology and sedimentology when establishing palaeoenvironmental interpretations from either core or outcrop based rock record datasets.

2) **Chapter three** investigates the early stage taphonomic processes that operate on unionid shells. It emphasises the close relationship between the unionid bivalves distinctive layered shell structure and the complex interplay of physically and chemically induced, as well as biogenically mediated decay processes. These factors were observed to act at both microscopic and macroscopic scales as documented by visual observation and SEM imaging. The taphonomic processes appear to be closely influenced by variations in the chemical and physical properties of the layered shell structure. Damage to the umbonal region of the shell is an obvious and distinctive feature and occurs initially during the life of

the bivalve. First the periostracum is punctured and torn by physical processes. Following exposure, the aragonitic portions of the shell are sculpted, resulting in the production of pitting and hole structures. As well as features ascribed a physical or chemical cause, these also include the creation of *Oichnus* like boring structures that may be of bacterial origin. Exposed conchiolin layers are exploited by biofilm and varied microorganisms that are both described at greater length in chapter four.

3) **Chapter four** explores a range of microboring structures encountered in unionid shells as revealed by SEM imaging directed at both shell samples and epoxy resin casts. A close relationship is demonstrated between these structures and the layered unionid shell structure, with prevalent boring in the nacreous aragonitic and associated conchiolin layers of the shell. Three main morphologies are represented, with simple, steeply aligned, sub-micron penetrative tubular borings spanning the entire shell thickness. More complex branching tubular boring networks are also present, with two to five micron diameter tubes. These tubular borings both occur in the nacreous aragonitic portion of the shell. These structures are likely a consequence of cyanobacteria, although based on morphology and dimensions they may also be derived from chlorophytes or fungi. In addition, a complex reticulated network is observed on a conchiolin layer within the nacreous portion of the shell. This structure has morphological similarity to some structures produced by Myxomycetes (social amoebae/slime moulds) and blends laterally into an amorphous extracellular polymeric substance (EPS) biofilm. It may be of fungal origin since Fungi are commonly associated with exploitation of organic substrates (e.g. Romaní, 2010). Organic conchiolin shell layers are frequently noted to be colonised by EPS based biofilms, alongside additional elements including bacteria, diatoms, sheets and filaments of fungal

or cyanobacterial origin and debris including nacre tablets and lithic fragments that are often found associated with and embedded in the biofilms. The variety of structures encountered suggests that boring activity may be a prevalent feature within freshwater settings and may represent a prolific area for future study in both modern day and rock record contexts.

4) **Chapter five** shows that thick epibiotic growths of microbial mat may develop on the exterior shell surfaces of unionid bivalves. Based on their colour, morphology and textural properties the mats are likely dominated by either cyanobacteria or chlorophytes. These may build on a foundation of biofilm and may also include diatoms (introduced and described in chapter four). The mat growths do not appear to interfere with the normal, healthy functioning of the bivalves. The extent and thickness of the microbial mats and their location relative to the sediment water interface suggest that full burial of the unionids and resultant sloughing of attached epibiota are not a characteristic feature of this unionid population. There remains a paucity of published geological or biological literature in the field of freshwater epibiota and this suggests that native epibiotic associations may be more ubiquitous in freshwater settings than previously documented.

5) **Chapter six** presents examples of the benign attachment of Trichoptera cases on unionid substrates. Selective preferential spatial attachment was observed across the shell surface, with Trichoptera universally attached to regions of pristine periostracum rather than the aragonitic and associated conchiolin shell layers. In combination with evidence from SEM observation of shell textures, this suggests that unionids possess neither chemical nor physical mechanisms intended to inhibit epibiotic biofouling. This contrasts with marine bivalves where

some taxa are noted to incorporate such defensive measures (Bers et al., 2010 and references therein). Investigation into the possible presence and distribution of these defences in freshwater settings represents a novel field of enquiry. The presence of a single Palaeocene fossil example (reported by Erickson, 1983) and overlap between the age ranges of unionids and case making Trichoptera suggest the potential for a mesozoic origin for this association. Given the prevalence of both fossil unionids and Trichoptera cases in isolation there may be other overlooked preserved examples of this association. If encountered, these could provide valuable information regarding seasonality of deposition as well as other palaeoenvironmental parameters.

RECOMMENDATIONS FOR FUTURE WORK

Given sufficient time and resources, there are several avenues via which the results of this study could be expanded:

1) Although there is no reason to suspect they are isolated instances, the ichnological and epibiotic associations explored herein are for the most part merely hinted at in the previously published literature. It would be of great value to our understanding of unionid ethology and ecology to determine the ubiquity or otherwise of the observations and a greater number of collection points would facilitate this. A larger data set may enable any trends associated with differences in environmental parameters to be displayed. In particular, comparison might be undertaken between river and lake localities. In addition, trends in a variety of factors such as substrate grain size, water energy levels, availability of nutrients and water chemistry and temperature could all influence the taphonomic signatures, epibiota and ichnological assemblages associated with unionid

populations.

2) Studies could be undertaken to demonstrate the factors influencing unionid locomotion and whether a static or motile habit is adopted, in both river and lake localities. In both environments, the unionids life position could be documented to determine azimuth relative to water currents, and both orientation and depth of penetration relative to the sediment-water interface. In addition, any trends in unionid trace orientation relative to water current could be recorded. The importance of substrate grain size and firmness as influencing factors could also be explored. Results of such study might have utility when interpreting palaeoenvironmental parameters of assemblages preserved in the rock record.

3) Chemical analysis and SEM imaging across a more taxonomically diverse array of unionids and other freshwater bivalves and gastropods could be undertaken to determine whether there are any freshwater molluscan taxa with either physical or chemical mechanisms in place intended to inhibit biofouling by attached epibiota.

4) In an extended study, applicable techniques could also be applied to more tightly constrain the taxonomy of the organisms associated with the biofilm, biomat and microboring activity.

5) To further document unionid taphonomy, experimental studies could be undertaken, via the introduction of pristine shell material in river or lake settings and documentation of the rates of decay through observations of the samples undertaken at known time intervals. Experimental work could also be undertaken on the development of the shell pitting structures, by lab based study of sediment abrasion under different flow conditions and observation of the resulting shell

surface textures. Actualistic study of taphonomic processes affecting caddisfly pupal cases could examine how long the cases might remain attached to a substrate after being vacated and also how quickly the case structures themselves will be destroyed. Processes operating prior to and following shallow burial could be studied in unionids and associated sediments from both Holocene deposits as well as in the older rock record to document both the probability of, and any features associated with, long term preservation. In addition to palaeoecological and palaeoenvironmental applications these observations might be of interest in archeological contexts.

CONCLUSION

The interdisciplinary study presented herein resulted in the following main contributions to knowledge of freshwater ichnology, taphonomy and epibiota:

1) That freshwater river locations may include complex molluscan derived trace assemblages, with both diverse and dense assemblages of macroscopic soft sediment traces being observed on the surface of the riverbed sediment. Furthermore, traditional established freshwater ichnofacies models may be incomplete insofar as they fail to address the subtleties of variations in trace assemblage observed in freshwater environments.

2) The layered shell structure of unionid bivalves exerts a strong influence on taphonomic processes, with distinct processes observed for the conchiolin and aragonitic portions of the shell.

3) That unionid bivalve shells represent a significant substrate for colonisation

and exploitation by other organisms, to a far greater degree than is suggested by a review of existing literature. This exploitation occurs at a number of scales and can be closely governed by shell structures, which display spatial variability as a consequence of and associated with taphonomic decay. This exploitation includes both epibiota and microboring.

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